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Minireview

Molecular Advances in Pretargeting Radioimunotherapy with Bispecific Antibodies[1]

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Abstract

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The use of antibodies against tumor-associated cell surface antigens for the targeted delivery of radionuclides was introduced >20 years ago. Although encouraging results have been achieved with radiolabeled antibodies in the management of hematopoietic malignancies, there remains a need for successfully treating solid tumors with this modality. One promising approach involving pretargeted delivery of radionuclides has been shown to be capable of significantly increasing the radioactive uptake in tumor relative to normal organs, thereby potentially improving the efficacy of both detection and therapy of cancer. Uncoupling of the radionuclide from the tumor-targeting antibody allows the relatively slow process of antibody localization and clearance to occur before a very rapid and highly specific delivery of the radioactive payload carried on a small molecule, such as a peptide. This minireview discusses the various strategies and advancements made since the concept of pretargeting was proposed in the mid-1980s, with emphasis on those comprising bispecific antibodies for cancer therapy. Critical aspects of these pretargeting systems for achieving higher tumor:nontumor ratios are considered. In addition, both preclinical and clinical results obtained from a pretargeting method known as the Affinity Enhancement System are presented. Future directions of pretargeting technology are also suggested.

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Targeted delivery of radionuclides for imaging and therapy of cancer, using antibodies against tumor-associated antigens or haptens, is an active area of ongoing investigation at both the experimental and the clinical levels (1). Since the introduction of the terms RAID[3] and RAIT, representing the use of radiolabeled antibodies for imaging and therapy, respectively, >20 years ago, the extensive efforts have resulted in the commercialization of several cancer-imaging agents based on RAID. The current prospects of establishing RAIT as a new therapeutic modality of cancer is also very promising because of the encouraging results obtained with radiolabeled antibodies in the management of hematopoietic neoplasms, especially non-Hodgkin's lymphoma (2).

In contrast to hematopoietic malignancies, solid tumors have been less responsive to RAIT. Because the efficacy of RAIT depends on several factors, including cumulative radiation dose delivered, dose rate, penetration, and tumor radiosensitivity, one major reason for the diminished success of RAIT in solid tumors may be the suboptimal tumor uptake of the targeting antibody. At an accretion of 0.001–0.01% ID of the radiolabeled antibody/gram tumor, a cumulative tumor dose of <1500 cGy is usually delivered, which falls short of the typical >5000 cGy needed to achieve therapeutic responses in most neoplasms, based on external beam irradiation of adenocarcinomas (3). When the accretion is limited, doses needed to achieve therapeutic response are restricted because of other normal organ dose limitations. For RAIT, bone marrow toxicity often determines the maximum tolerated dose that can be administered.

The challenge of treating solid tumors by RAIT has stimulated a number of approaches to improve the radiation dose delivered and to achieve a more uniform distribution of ionizing radiation, with the ultimate goal being the delivery of tumoricidal doses while sparing normal tissues. One of these methods is pretargeting, a strategy conceived in the 1980s initially for improving the selective delivery of radionuclides by antibodies to tumors and later extended to include nonradioactive agents and nonantibody delivery systems. This minireview addresses the various refinements of the pretargeting strategy, with emphasis on those involving the use of bsAb and radioactive agents in the therapy of cancer. The progress of pretargeting research in the realm of nuclear medicine has received periodic appraisals over the years through excellent editorials (4–8) and reviews (9–15).

Approaches Taken to Improve Target:Nontarget Ratios

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The inherent incompatibility between a tumor-targeting antibody and the radiation carried by it became evident from early imaging studies in experimental animals and in patients. Intact immunoglobulins directly labeled with a radioisotope were found to clear very slowly from the circulation, resulting in high background activity, thereby limiting the efficacy for both the detection and treatment of tumors (4). The use of antibody fragments, such as Fab or F(ab')$_2$, which clear from the blood more rapidly than whole IgG, improves target:background ratios but at the expense of lower uptake of the radiolabeled antibody in the tumor, when compared with whole IgG (16). Other approaches to reduce background radioactivity while maintaining tumor uptake of a directly radiolabeled antibody agent have been explored and met with variable degrees of success, *e.g.*, administration of antiantibodies (such as an anti-idiotype antibody) can remove radiolabeled antibodies from blood faster than from tumor and is considered particularly applicable to radioiodinated antibodies (17). For antibodies labeled with a radiometal, the insertion of a metabolizable linker between the chelate and protein is also effective in improving tumor:background ratios, despite a modest drop in tumor concentrations (18). The fact that an antibody can be facilely labeled *in vivo* with a radioactive hapten to which it binds with a high affinity is fundamental to the development of pretargeting strategies, which successfully overcome the problem of low target:background ratios by temporal separation of the slow antibody-targeting step from the delivery of the radionuclide.

Principles, Strategies, and Key Components of Pretargeting

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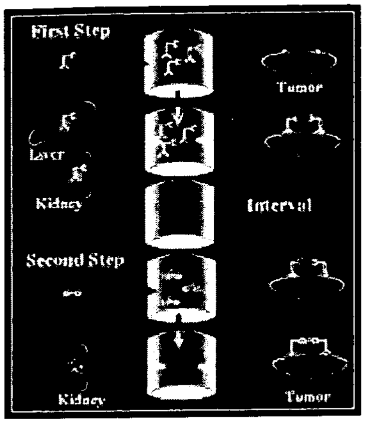
The essence of pretargeting involves the use of a macromolecule that is capable of binding with a high affinity to a radioactive agent of low molecular weight (usually M_r <10,000) and can also selectively target a tumor antigen. The macromolecule is administered first, and the radioactive agent ("the effector") is given at a later time, ideally when the concentration of the macromolecule in the tumor is greater than in other tissues. Favorable tumor:normal tissue ratios of radioactivity are thus achievable, because the small size of the nontargeted radioactive agent permits its rapid elimination from the body. This sequence of events is commonly referred to as the two-step protocol (Fig. 1). The key for the success of the two-step method is that the macromolecule must be cleared sufficiently from the blood and normal tissues, otherwise the radioactive effector molecule will be retained wherever the macromolecule is distributed. An alternative to the two-step protocol is the three-step protocol, in which a specific molecule ("the chaser") is injected to remove the residual macromolecule from the bloodstream before giving the radioactive agent, so that further enhancement in the tumor:blood ratios of radioactivity can be obtained. To date, the potential value of applying pretargeting strategies to cancer imaging and therapy has been demonstrated in animal models, as well as in clinical trials for many macromolecule/effector systems, with or without the chase step, using radioactive or nonradioactive effectors (12, 14, 19).

Fig. 1. The two-step pretargeting protocol illustrated with a bsAb and a bivalent hapten.



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To be useful for pretargeting applications, the macromolecule can be just an antihapten monoclonal antibody that recognizes a structural component (hapten) of an effector (20) or even merely a biotin-binding protein like streptavidin (21–23). These molecules can be used because tumors or inflammatory/infectious foci, because of their leaky capillaries, can accumulate macromolecules nonspecifically through passive diffusion, leading to a preferential localization of the macromolecule at the target. In most cases studied, however, the macromolecule of interest consists of a modified antitumor antibody carrying a secondary recognition moiety, in addition to its primary target-binding function. Representative examples of such modified antibodies can be distinguished into five types: (*a*) bsAb (24) constructed to contain one hapten-binding site and one or two target-binding sites; (*b*) antibodies conjugated to streptavidin (25) or avidin (26) to enable the binding of biotin; (*c*) biotinylated antibodies capable of complexing with avidin (27) or streptavidin (28, 29); (*d*) antibodies conjugated to DNA (30) to promote binding to complementary nucleotide sequences; and (*e*) antibodies conjugated to enzymes (19) to activate a prodrug at the target site. In general, for each targeting macromolecule selected, a series of effectors may be devised and evaluated under pretargeting conditions for their respective uptake in tumor relative to normal tissues. These effectors, while having the common hapten or structural component for binding to the macromolecule, could differ in other attributes, such as hapten valency (31, 32), lipophilicity (33), dissociation rate (34), *in vivo* stability (35), binding affinity (36), and optical property (37), each of which may affect the distribution of the effectors in target as well as in normal organs.

A particular attribute that has proved to be influential on the tumor uptake of an effector is hapten valency. Effectors that contain a bivalent hapten are better tumor localizers than their monovalent analogs. The enhancement is believed to be because of the ability of the bivalent hapten to cross-link the pretargeted macromolecule at the tumor site, resulting in the formation of a more stable complex (Fig. 2) and, therefore, a longer tumor residence time. Bivalent haptens pretargeted with bsAb have been patented as the AES (38). As shown in the autoradiographs of Fig. 3, the clear tumor-to-nontumor contrast provided by the AES pretargeting method in an animal model with human small cell lung carcinoma expressing NCAM with a bispecific anti-NCAM x anti-HSG conjugate (NK1NB11 x 679, Fab‘ x Fab‘) and a radioiodinated (^{125}I) di-HSG hapten is striking, compared with that obtained from a directly radiolabeled anti-NCAM antibody (39). Excellent images were also obtained (40) in nude mice bearing human renal cell carcinoma xenografts, by pretargeting with a bispecific antirenal cell carcinoma x anti-indium-DTPA conjugate (G250 x 734, Fab‘ x Fab‘) for 72 h followed by ^{111}In-labeled di-DTPA hapten (Fig. 4). Clinical trials (41) using the AES pretargeting method with a bispecific anti-CEA x anti-indium-DTPA conjugate (hMN14 x 734, Fab‘ x Fab‘) and a radioiodinated (^{131}I) di-indium-DTPA hapten (Pentacea; IBC Pharmaceuticals, Morris Plains, NJ) also produced impressive images of tumor uptake with high target-to-background contrast, as shown in Figs. 5 and 6 for two patients with CEA-expressing cancers.

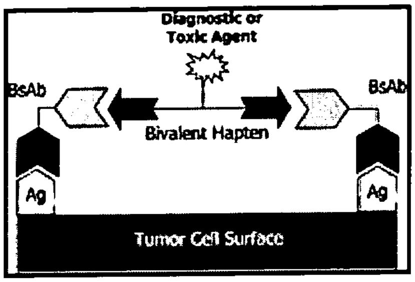

Fig. 2. The AES showing the cross-linking of 2 bsAb on the targeted tumor surface with one bivalent hapten. The bivalent hapten can carry a diagnostic or toxic agent.

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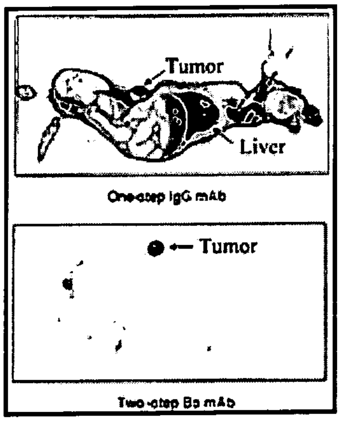

Fig. 3. Autoradiographs of mice bearing National Cancer Institute-H69 tumor obtained with a directly radiolabeled (^{125}I) N1KNBL1 (*top panel*) or a radiolabeled (^{125}I) di-HSG hapten pretargeted with bispecific NK1NBL1 x 679 for 48 h (*bottom panel*). Both autoradiographs were taken at 48 h after injection of the respective radiolabeled agents (from Ref. 39, with permission).

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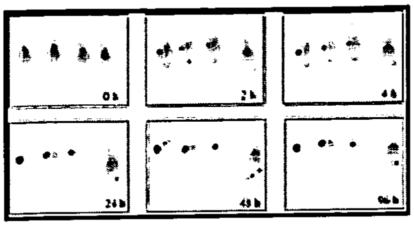
Fig. 4. Whole body images of NU-12 tumor-bearing mice targeted with a directly radiolabeled (^{111}In) monoclonal antibody G250 or a radiolabeled (^{111}In) di-DTPA hapten administered 3 days after the bsAb G250 x DTIn1. Each



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frame shows one mouse (*far right image*) using the one-step approach and three mice (the remaining three images) using the two-step pretargeting approach, at the indicated time points. The location of tumor was indicated with an *arrow* in the 48-h frame (from Ref. 40, with permission).

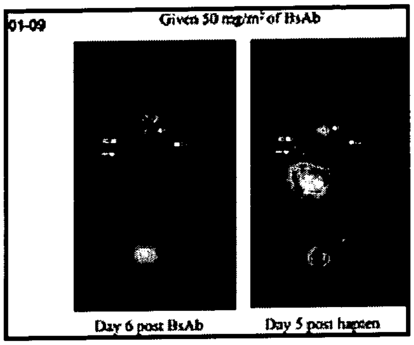

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Fig. 5. Anterior views of a patient (*01–09*) with rectum carcinoma on day-6 postinjection of ^{131}I-labeled hMN14 x 734 (50 mg/m^2, 10 mCi, *left panel*) and on day-5 postinjection of ^{131}I-labeled Pentacea (100 mCi, 70 μg, *right panel*). The hapten was injected 7 days after the bsAb. The malignant lesions, as documented by computed tomography of the chest, lungs, and liver, were revealed in both images (indicated by *arrows* for those in the chest and lungs), but the contrast was much greater with the hapten.

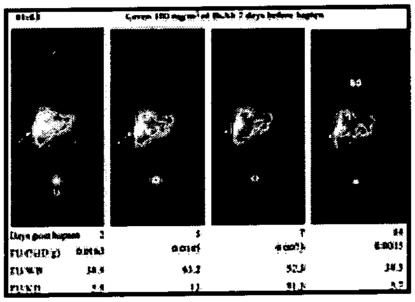

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Fig. 6. Anterior views of a patient (*01–13*) with small cell lung carcinoma on days 2, 5, 7, and 14 postinjection of ^{131}I-labeled Pentacea (100 mCi, 70 μg). The hapten was injected 7 days after the bsAb (100 mg/m^2). The multiple metastases, as documented by computed tomography in the liver, were remarkably shown in all images. The %ID/gram in the tumor (*TU*), the ratio of tumor:whole body (*TU: WB*), and the ratio of tumor:kidney (*TU:KD*) were measured for the hottest and largest tumor in the external part of the right lobe (*arrow*). The calculated values were shown for each time point under the respective panels.

The different pretargeting systems that have been investigated are grouped into nine categories in Table 1 according to the constituent of the targeting macromolecule. Within each assigned category, they may be further differentiated with respect to the following parameters: molecular size of the macromolecule, molecular form of the antibody component comprising the macromolecule, method of preparing the macromolecule or source of the macromolecule, valency of the macromolecule to the target, valency of the macromolecule to the hapten or biotin, hapten valency of the effector to the macromolecule, and other functional groups carried by the effector. The search for an effective pretargeting system worthy of commercialization necessitates continuous optimization of several or all of these parameters.

View this table: *Table 1* Pretargeting systems investigated^{a}
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Early Demonstration of Pretargeting Concept

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Before the term "pretargeted immunoscintigraphy" first appeared in publication (44) in 1987, the feasibility of using a bsAb or an antihapten antibody to increase the tumor concentration of diagnostic imaging agents had been assessed in several seminal studies with encouraging results (45–47). These earlier achievements were facilitated by the availability of a murine monoclonal antibody, CHA255, having a high binding affinity (Kd = 10^{-9} to 10^{-10} M) for indium benzyl EDTA (48) and a synthetic bsAb, ZCE x CHA (49), prepared by linking the Fab' of an anti-CEA murine monoclonal antibody (ZCE025) with the Fab' of CHA 255. Coadministration of ^{111}In benzyl EDTA or derivatives of ^{111}In benzyl EDTA containing cobalt-bleomycin (BLEDTA II or BLEDTA IV) with a sufficient amount of CHA255 was found to alter profoundly the pharmacokinetics of these small molecules in tumor-bearing mice (45). Specifically, with CHA255 acting as an *in vivo* carrier, the biological half-life of these small molecules was increased from minutes to days, and the concentrations of radioactivity in all organs, most notably tumor and blood, were markedly higher, when measured at 24 h. Furthermore, the injection of a "flushing" dose of unlabeled indium benzyl EDTA was effective at reducing more radioactivity in blood than in tumor, resulting in as much as a 50-fold increase in the tumor:blood ratio (from 0.8 to 47.1 for ^{111}In-benzyl EDTA) within 3 h. Comparable improvements (46, 47) in tumor:background ratios were observed in tumor-bearing mice for ZCE x CHA, using ^{111}In benzyl EDTA or ^{111}In BLEDTA IV premixed with a finite amount of ZCE x CHA as a carrier to ensure a higher tumor uptake. In an optimized protocol in which 14 µg of the synthetic bispecific Fab' x Fab' conjugate were allowed to prelocalize for 24 h before injecting 10µCi of ^{111}In BLEDTA IV premixed with 3 µg of ZCE x CHA, the concentration of radioactivity in tumor at 24 h was 7% ID/gram (50), with the tumor:blood ratio being ~5. Presumably, transferring ^{111}In BLEDTA IV from circulating ZCE x CHA to prelocalized ZCE x CHA at the tumor had occurred efficiently to account for the observed *in vivo* targeting. Additional biodistribution studies (33, 50) undertaken to compare BLEDTA IV with a series of benzyl EDTA analogs concluded that ^{111}In hydroxyethylthiourea-benzyl-EDTA, while showing similar uptake in normal tissues as ^{111}In BLEDTA IV, had a remarkable increase in tumor concentration, topping 18% ID/gram at 24 h (50).

Critical Factors of Pretargeting

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As the general principles of pretargeting were being demonstrated, a number of key factors that could affect the tumor-targeting potential of a hapten-antibody delivery system was also elucidated. Subsequent efforts to address these factors to the advantage of pretargeting applications have led to various refinements of the original approach exemplified by ZCE x CHA and ^{111}In BLEDTA IV (42).

Binding Constant.

A higher affinity of hapten for antibody is important to pretargeting, because it would enhance the noncovalent association between antibody and hapten, thus reducing the amount of antibody required for capturing hapten at the target site. Because binding constants of antibodies rarely exceed 10^{-10} M, additional improvements coming from binding constants would have to rely on other existing noncovalent systems that display affinities much higher than 10^{-10} M. Accordingly, the very high affinity of biotin for avidin or streptavidin ($K_d = 10^{-15}$ M, about six to seven orders of magnitude above the binding constants usually achievable with antibodies) was distinguished immediately, and the suitability of biotin-avidin or biotin-streptavidin for pretargeting applications was quickly established (26). Pretargeting systems based on the binding of biotin to avidin or streptavidin have been developed, and some tested extensively in the clinic (27, 51, 52). The simplest system involves only streptavidin and a labeled biotin-chelate conjugate. Pretargeting with streptavidin followed by ^{111}In labeled EDTA-hydrazino-biotin was shown to be useful for imaging infectious lesions in a mouse model (21). Other systems are more elaborate and may consist of one of the following: (*a*) pretargeting with a streptavidin-antibody conjugate followed by a radiolabeled biotin-chelate (25); (*b*) pretargeting with a streptavidin-antibody conjugate followed by a clearing agent and then a radiolabeled biotin-chelate (51–55); (*c*) pretargeting with an avidin-antibody conjugate followed by a radiolabeled biotin-chelate (26); (*d*) pretargeting with a biotin-antibody conjugate followed by a radiolabeled streptavidin (29); (*e*) pretargeting with a biotin-antibody conjugate followed by avidin and then a radiolabeled biotin-chelate (56); and (*f*) pretargeting with a biotin-antibody conjugate followed by avidin, then streptavidin, and finally a radiolabeled biotin-chelate (57). Thus, procedures can consist of two, three, or more steps, all intended to increase tumor:nontumor ratios. However, both endogenous biotin and the immunogenicity of avidin and streptavidin can affect the results (54, 58). In addition to (strep)avidin–biotin, other recognition systems conferring high specificity and affinity, either based on complementary oligonucleotide binding (30) or enzyme-substrate interaction (19), have also been adapted for pretargeting applications. The preference of higher affinity also applies to the binding of antibody to target antigen. For a bsAb (monovalent Fab' x monovalent Fab') with a K_d for target antigen already in the 10^{-9} to 10^{-10} M range, additional improvements may still be possible by incurring bivalent binding with a construct like $F(ab')_2$ x Fab' or an engineered bispecific protein preserving the bivalency for the target antigen.

Hapten Valency.

Compared with their monovalent analogs, bivalent haptens have consistently shown higher tumor uptake, a phenomenon attributed to cross-linking antibodies that are specifically bound to tumor surface or nonspecifically accumulated in tumor. Bivalent haptens are used today in almost all pretargeting systems, including those involving biotin/(strept)avidin. The binding between a DNA antibody and its antisense oligonucleotides can also be regarded as multivalent. A variant of bivalent hapten is the so-called asymmetric bivalent hapten (59), which contains two different haptens on the same molecule, with each hapten suitable for binding to a different antihapten antibody. Asymmetric bivalent haptens were designed to increase the targeting specificity when used in conjunction with two distinct bsAb, which differ from each other in antigen, as well as hapten specificity (60). This triple-component system for pretargeting would ensure that only cells expressing double antigens could have accrued both bsAb, which in turn could be cross-linked only by a relevant asymmetric hapten. The advantage of bivalency also applies to the binding of antibodies to target antigen. A modified antibody capable of binding bivalently to target antigen is likely to show an increase in target residence time, which may be further extended by using a relevant bivalent hapten.

Specific Activity.

The amount of macromolecule available for capturing hapten at the target site is usually very small (in the order of nmol/gram tumor in human subjects). Consequently, the amount of hapten deliverable to the target is quite limited. With a hapten labeled at a higher specific activity, increased radiation dose can be imparted per unit mass to effect more damage or easier detection of the target, thereby improving pretargeting efficacy. Specific activities not <1 mCi/nmol was thought necessary for good pretargeting results (6). In clinical studies, biotin-chelates or hapten-chelates labeled to specific activity ranging from 0.05 to 1.5 mCi/nmol have been acceptable.

Two factors pursuant to specific activity are choice of radionuclide and chelate. Radionuclides that have been evaluated for pretargeting applications include ^{111}In and ^{99m}Tc for single photon imaging (20–22, 25–27, 31, 32, 42, 50); ^{68}Ga for PET imaging (37, 61, 62); ^{131}I, ^{90}Y, and ^{188}Re for ß-particle therapy (36, 51–53, 63–68); and ^{212}Bi for α-particle therapy (13). Table 2 lists several physical features (half-life, energy of emission, type of emission, maximum particle range, and production mode) of radionuclides that are of current interest for RAIT. Additionally listed in Table 2 are characteristic chelating agents capable of forming stable *in vivo* complexes with each radiometal. For α-emitters, more work may be needed to develop better strategies or chelates for binding them in a stable manner. The subjects of therapeutic radionuclides and choices of their chelates have been reviewed elsewhere (69, 70).

View this table: *Table 2* Radionuclides of current interest for RAIT
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Preferably, a therapeutic radionuclide selected for use with a particular pretargeting system should possess the following properties: (*a*) a half-life similar to that of the radiolabeled hapten-chelate at the target site; (*b*) the ability to discharge particles of higher energy for effective cell killing; (*c*) the availability of a suitable hapten-chelate for efficient labeling and the resultant formation of a stable *in vivo* complex that exhibits rapid diffusion, little uptake in nontarget tissues, and fast clearance; and (*d*) the feasibility of production with a generator after easy elution. The most prevalent radionuclide used for therapy in clinical trials today is still ^{131}I, despite its relatively long half-life (8 days), suboptimum energy of ß-particle, and concomitant emission of γ rays, which requires isolation of patients in shielded rooms for several days but provides the benefit of imaging. Compared with ^{131}I, ^{90}Y is almost ideal for therapy with pretargeting approaches, because: (*a*) it has a half-life (67 h) similar to that of the radiolabeled effector after prelocalization of the targeting macromolecule; (*b*) it discharges only electrons of higher energy to cause deeper tissue penetration; (*c*) it can be labeled efficiently with DOTA-bearing haptens, forming a stable *in vivo* complex that exhibits satisfactory pharmacokinetics; and (*d*) it can be produced by a generator and available commercially with acceptable purity. Other therapeutic radionuclides with one or more favorable physical properties than ^{131}I are ^{188}Re and several α-emitters under active development. Like ^{90}Y, ^{188}Re is available carrier free and, except for a shorter half-life (17 h), has three of the four attributes characteristic of ^{90}Y as a promising therapeutic radionuclide. It is noted that short-lived radionuclides ($t_{1/2}$ about hours), α-emitters in particular, are necessarily restricted to rapid targeting processes, which are feasible with pretargeting approaches because the radiolabel resides on a fast-diffusing, small molecule. Radioisotopes emitting α-particles offer several advantages over ß-emitters for therapy: (*a*) discharged α-particles with a mass 8,000 times greater than ß-particles exhibit high linear energy transfer (100 KeV/μm) and are therefore very cytotoxic, requiring as few as 6 or 7 disintegrations for internalized α-emitters and ~25 disintegrations for surface-bound α-emitters to kill a cell; (*b*) the extremely short path length (40–80 μm) of α-particles greatly reduces the nonspecific irradiation of normal tissue around the target cell; (*c*) the DNA damage caused by α-particles is not easily repaired by the cell; and (*d*) the cytotoxicity of α-particles is not affected by oxygen, and, therefore, cell killing is effective under hypoxic conditions usually existing in established tumors. However, the lack of suitable chemistry or effective chelates for preparing stably attached α-emitters has hampered their further development as agents for pretargeting applications. Table 3 compares the dosimetry data obtained from animal studies with ^{131}I, ^{90}Y, and ^{188}Re under specified pretargeting conditions, as reported in a limited number of publications (39, 64, 66–68). In each case, the tumor was shown to receive a radiation dose considerably higher than any of the normal organs.

View this table: *Table 3* Dosimetry data obtained in animal models with pretargeting
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In addition to those discussed above, numerous other factors have also been considered as important for improving pretargeting efficacy, *e.g.*, issues like molecular size, immunogenicity, target specificity, antigen density, and antigen modulation should be carefully considered in the design and construction of ultimate targeting macromolecules. As for the hapten-chelates or biotin-chelates, molecular size, immunogenicity, clearance rate, elimination route, and *in vivo* stability deserve proper attention. Pretargeting systems using a chase step may further face optimization issues involving the chase molecule, such as molecular size, valency, immunogenicity, clearance rate (71), and elimination route.

Recent Progress and Future Directions

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The success of receptor targeting by peptides, as demonstrated by the regulatory approvals of peptide imaging agents, such as ^{111}In-DTPA-pentatreotide for human neuroendocrine tumors, has prompted the adaptation of peptides for pretargeting approaches. One possible system described by Goodwin and Meares (5) involves the conjugation of receptor-specific peptides to long-circulating PEG, which is also derived with an avidin or streptavidin for the recognition by a radiolabeled DOTA-biotin dimer. By using PEG with branched structures, multiple peptides and other optional agents can be attached, along with avidin or streptavidin, thereby yielding a targeting macromolecule with many of the desirable features, including multivalency for the target (through multiple peptide), multivalency for the effector (through multiple binding sites on avidin/streptavidin for biotin), reduced immunogenicity (via PEG), prolonged circulation to reach high target concentration (via PEG of suitable molecular size), and the option of rapid clearance with the use of a biotin-PEG conjugate, which should be nonimmunogenic.

The trend at using smaller antibody fragments for *in vivo* applications has spurred the development of bispecific diabody or bispecific tetrabodies suitable for pretargeting approaches. We have produced two bispecific diabodies (BS1.5 and BS1.5H) for pretargeted delivery of radiolabeled bivalent haptens to tumors expressing CEA (43). BS1.5 (M_r ~54,000) consists of two heterologous polypeptide chains associated noncovalently to form one binding site for CEA from the variable domains of hMN14 (a humanized anti-CEA antibody from Immunomedics, Inc., Morris Plains, NJ) and one binding site for HSG from the variable domains of 679 (a murine monoclonal antibody specific for HSG). In BS1.5H, humanized 679 V_H and V_K domains were used. The V domains were engineered into a dicistronic *Escherichia coli* expression vector for directing the synthesis of the two polypeptides, 679V_H-GGGGS-hMN14V_K-6His and hMN14V_H-GGGGS-679V_K-6His, in the periplasmic space of *E. coli*. The diabody was purified from the soluble fraction of isopropyl-1-thio-ß-D-galactopyranoside-induced *E. coli* culture by Immobilized Metal Ion Affinity Chromatography and ion exchange chromatography. Kinetic analyses on BIAcore using HSG-immobilized sensor chips showed that the binding affinity for HSG was similar for BS1.5, BS1.5H, and chemically linked hMN14 x 679 (M_r ~100,000, Fab' x Fab'). The bispecificity of BS1.5 or BS1.5H was demonstrated on BIAcore by measuring the additional increase in response units on successive injections of the bispecific diabody followed by CEA. The utility of BS1.5 for tumor pretargeting was evaluated in CEA human tumor-bearing mice using a bivalent HSG hapten (IMP-241) labeled with ^{111}In, and the results obtained were compared with those of hMN14 x 679. For BS1.5 injected 8 h before giving the hapten, tumor uptake of ^{111}In was 10.3 and 6.3% ID/gram at 3 and 24 h, respectively, with the tumor:blood ratios being 167 at 3 h and 631 at 24 h. For hMN14 x 679 injected 24 h before giving the hapten, the tumor uptakes of ^{111}In were 11.3 and 6.9% ID/gram at 3 and 24 h, respectively, with the tumor:blood ratios being 8.1 at 3 h and 16.4 at 24 h. Thus, the concentrations of hapten in the tumor were similar between BS1.5 prelocalized for 8 h and hMN14 x 679 prelocalized for 24 h. However, because the radioactivity in all nontumor organs was higher for hMN14 x 679, the tumor:nontumor ratios were superior for BS1.5. These results, as summarized in Table 4, indicate that BS1.5 is an attractive candidate for use in a variety of pretargeting applications.

View this table: *Table 4* Biodistribution[a] of ^{111}In-IMP241 with pretargeted BS1.5 *vs.* hMN14
[in this window] x 679
[in a new window]

The potential of using a radiosensitizer to augment the therapeutic efficacy of RAIT delivered by the AES pretargeting method has also been demonstrated. In one study (72), paclitaxel, but not doxorubicin, was shown to improve the antitumor response in animals given an anti-CEA, anti-indium-DTPA bsAb (F6 x 734), followed by ^{131}I-labeled bivalent hapten and the chemotherapeutic drugs. A synergistic effect was observed without increasing toxicity.

These promising developments seem to indicate that the future evolution of pretargeting technology will be directed to at least three potential approaches. The first is to use peptides instead of antibodies for specific targeting. The second is to replace chemically linked bsAb conjugates with recombinant fusion proteins of multispecificity and multivalency. The third is to investigate whether a certain chemotherapeutic agent, which is also a known radiosensitizer, can produce synergistic effects when combined with a pretargeting system for treating a particular cancer. In addition, the potential of a hapten or hapten-chelate that also contains a receptor-binding moiety for enhancing the specific uptake in receptor-positive target cells is worth exploring. Needless to say, the urgent need to establish, for each pretargeting system in carefully designed clinical trials, the optimal amount of the targeting macromolecule, the best timing of the second injection with the effector ("two-step protocol") or the chaser ("three-step protocol"), the maximum tolerated dose, and the therapeutic efficacy for a certain indication continues to exist and should serve as a basis of future experimentation.

Footnotes

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[3] The abbreviations used are: RAID, radioimmunodetection; RAIT, radioimmunotherapy; ID, injected dose; AES, affinity enhancement system; NCAM, neural cell adhesion molecule; HSG, histamine-succinyl-glycine; DTPA, diethylenetriaminepentaacetic acid; CEA, carcinoembryonic antigen; DOTA, 1,4,7,10-tetra-azacylododecane-*N, N',N'',N'''*-tetraacetic acid; TETA, 1,4,8,11-tetraazacyclotetradecane-*N, N',N'',N'''*-tetraacetic acid; CHX-A-DTPA, stereoisomers of cyclohexyl-DTPA; PEG,

polyethyleneglycol; bsAb, bispecific antibody.

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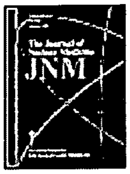

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J. Nucl. Med., January 1, 2005; 46(1_suppl): 115S - 127S.

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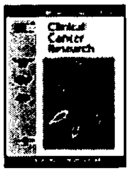

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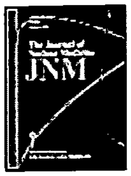
T. M. Cardillo, H. Karacay, D. M. Goldenberg, D. Yeldell, C.-H. Chang, D. E. Modrak, R. M. Sharkey, and D. V. Gold

Improved Targeting of Pancreatic Cancer: Experimental Studies of a New Bispecific Antibody, Pretargeting Enhancement System for Immunoscintigraphy

Clin. Cancer Res., May 15, 2004; 10(10): 3552 - 3561.

[Abstract] [Full Text] [PDF]



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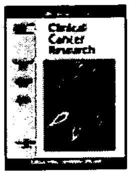
G. L. Griffiths, C.-H. Chang, W. J. McBride, E. A. Rossi, A. Sheerin, G. R. Tejada, H. Karacay, R. M. Sharkey, I. D. Horak, H. J. Hansen, and D. M. Goldenberg

Reagents and Methods for PET Using Bispecific Antibody Pretargeting and 68Ga-Radiolabeled Bivalent Hapten-Peptide-Chelate Conjugates

J. Nucl. Med., January 1, 2004; 45(1): 30 - 39.

[Abstract] [Full Text] [PDF]



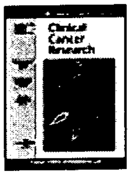
Clinical Cancer Research ▸HOME

R. M. Sharkey, H. Karacay, H. Richel, W. J. McBride, E. A. Rossi, K. Chang, D. Yeldell, G. L. Griffiths, H. J. Hansen, and D. M. Goldenberg

Optimizing Bispecific Antibody Pretargeting for Use in Radioimmunotherapy

Clin. Cancer Res., September 1, 2003; 9(10): 3897S - 3913.

[Abstract] [Full Text] [PDF]



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Pharmacokinetics and Dosimetry Studies for Optimization of Anti-Carcinoembryonic Antigen x Anti-Hapten Bispecific Antibody-mediated Pretargeting of Iodine-131-labeled Hapten in a Phase I Radioimmunotherapy Trial

Clin. Cancer Res., September 1, 2003; 9(10): 3973S - 3981.

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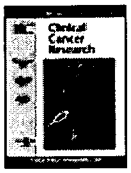
Clinical Cancer Research ▸HOME

M. D. Winthrop, S. J. DeNardo, H. Albrecht, G. R. Mirick, L. A. Kroger, K. R. Lamborn, C. Venclovas, M. E. Colvin, P. A. Burke, and G. L. DeNardo

Selection and Characterization of Anti-MUC-1 scFvs Intended for Targeted Therapy

Clin. Cancer Res., September 1, 2003; 9(10): 3845S - 3853.

[Abstract] [Full Text] [PDF]



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E. A. Rossi, R. M. Sharkey, W. McBride, H. Karacay, L. Zeng, H. J. Hansen, D. M. Goldenberg, and C.-H. Chang

Development of New Multivalent-bispecific Agents for Pretargeting Tumor Localization and Therapy

Clin. Cancer Res., September 1, 2003; 9(10): 3886S - 3896.

[Abstract] [Full Text] [PDF]

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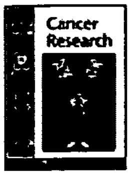
R. M. Sharkey, W. J. McBride, H. Karacay, K. Chang, G. L. Griffiths, H. J. Hansen, and D. M. Goldenberg



A Universal Pretargeting System for Cancer Detection and Therapy Using Bispecific Antibody
Cancer Res., January 15, 2003; 63(2): 354 - 363.
[Abstract] [Full Text] [PDF]

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=> s bispecific antibod?
L1         4142 BISPECIFIC ANTIBOD?

=> s l1 and multidrug resistant transporter
L2            0 L1 AND MULTIDRUG RESISTANT TRANSPORTER

=> s l1 and mutidrug transporter
L3            0 L1 AND MUTIDRUG TRANSPORTER

=> s l1 and transporter
L4            4 L1 AND TRANSPORTER

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L5            3 DUP REMOVE L4 (1 DUPLICATE REMOVED)

=> d l5 1-3 cbib abs

L5   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017  Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
AB   Immunoconjugates of a diagnostic agent and an antibody component that
     binds an epitope of a multidrug transporter protein are
     disclosed.  Thesse immunoconjugates are used in in vivo diagnostic methods
     to determine whether the failure of traditional chemotherapy is due to the
     presence of multidrug resistant tumor cells, multidrug resistant
     HIV-infected cells or multidrug resistant infectious agents.

L5   ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658  Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer
     treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
     targeted to multidrug resistant Pseudomonas aeruginosa were also prepared

L5   ANSWER 3 OF 3  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN                                               DUPLICATE 1
95328704  EMBASE  Document No.: 1995328704. Intracellular uptake and catabolism
     of anti-IgM antibodies and bi-specific antibody-targeted hapten by
     B-lymphoma cells. Manetti C.; Le Doussal J.M.; Rouvier E.; Gruaz-Guyon A.;
     Barbet J.. IMMUNOTECH, Imaging and Therapeutics Department, 130 Avenue de
     Lattre de Tassigny,13276 Marseille Cedex 9, France. International Journal
     of Cancer  63/2 (250-256)  1995.
     ISSN: 0020-7136. CODEN: IJCNAW. Pub. Country: United States. Language:
     English. Summary Language: English.
AB   The efficiency of radioimmunotherapy with iodine-labelled antibodies is
     often limited by intracellular internalisation and catabolism after
     initial binding to the cellular targets. We have developed a technique
     called affinity enhancement system (AES) which uses bi-specific antibodies
     to target radiolabelled bivalent haptens to cells. This targeting method
     has been applied successfully to tumour imaging in colorectal cancer
     patients and is now considered for therapy. We have investigated the
     potential of this technique to target iodine radioisotopes by comparing it
     to targeting with covalently iodinelabelled antibodies in a rapidly
     internalising antigenic system, the surface IgM of a B-lymphoma cell line.
     A 5-fold increase in the intracellular retention time of activity as
     compared to 125I-labelled F(ab')2 or IgG was observed. The radiolabelled
     hapten did not undergo any catabolism after internalisation. Resistance to
     cellular proteases and failure of recognition of the hapten by amino acid
     transporter systems may be potential explanations for these
     observations. This should make noncovalent targeting, particularly the
     AES, a method of choice to target modulating antigens for the therapy of
     malignant hemopathies.


=> s l1 and P-glycoprotein
L6          29 L1 AND P-GLYCOPROTEIN

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L7            14 DUP REMOVE L6 (15 DUPLICATES REMOVED)

=> s l7 and tumor
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L9   ANSWER 1 OF 10     MEDLINE on STN
2004091606.    PubMed ID: 14981526.    Efficient inhibition of
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multidrug-resistant human **tumors** with a recombinant bispecific anti-**P-glycoprotein** x anti-CD3 diabody. Gao Y; Xiong D; Yang M; Liu H; Peng H; Shao X; Xu Y; Xu C; Fan D; Qin L; Yang C; Zhu Z. (State Key Laboratory of Experimental Hematology, Institute of Hematology, Chinese Academy of Medical Sciences and Peking Tianjin Union Medical College, Tianjin, China.) Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K, (2004 Mar) 18 (3) 513-20. Journal code: 8704895. ISSN: 0887-6924. Pub. country: England: United Kingdom. Language: English.

AB Overexpressing of **P-glycoprotein** (Pgp) has been shown to be responsible for cancer resistance to multiple chemotherapeutic agents. Immunotherapy with biological agents, such as **bispecific antibodies** (BsAbs), may represent a promising approach to overcome the emergence of drug resistance. Here we constructed a recombinant BsAb, a diabody, with specificities to both CD3 on human T-lymphocyte and Pgp on cancer cells. The diabody was produced in Escherichia coli in a soluble functional form and purified by an affinity chromatography with a yield of >4 mg/l culture medium in shaker flask. The diabody binds to both CD3 on T-lymphocytes and Pgp on multidrug-resistant (MDR) **tumor** cells with affinities that are comparable to its respective parental single chain Fv molecules. In the presence of activated human peripheral blood lymphocytes (PBLs), the diabody mediates effectively the lysis of the Pgp-overexpressing human leukemia K562/A02 and epidermoid carcinoma KBv(200) cells, but is much less potent in mediating the lysis of the parent K562 and KB cells. Further, the diabody localized selectively within the K562/A02 xenografts in mice. When combined with activated PBL, the diabody significantly inhibited the growth of K562/A02 and KBv(200), but had no effect on K562 and KB xenografts. In contrast, treatment with doxorubicin, a standard chemotherapeutic agent, only inhibited the growth of K562 and KB, but had no effect on K562/A02 and KBv(200) xenografts. Taken together, our results suggest that the anti-Pgp x anti-CD3 diabody may have a great potential in the treatment of various MDR cancers.

L9 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN

2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug transporter protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant **tumor** cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

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2000:10868 Document No.: PREV200000010868. **Bispecific antibodies**, methods of production and uses thereof. Ring, David [Inventor, Reprint author]; Shi, Tian-Xiang [Inventor]. Redwood City, CA, USA. ASSIGNEE: Chiron Corporation. Patent Info.: US 5959084 Sep. 28, 1999. Official Gazette of the United States Patent and Trademark Office Patents, (Sep. 28, 1999) Vol. 1226, No. 4. print. CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

L9 ANSWER 4 OF 10 MEDLINE on STN

1998379107. PubMed ID: 9713485. Multidrug resistance and its reversal.

Volm M. (Department of Oncological Diagnostics and Therapy, German Cancer Research Center, Heidelberg, Germany.) Anticancer research, (1998 Jul-Aug) 18 (4C) 2905-17. Ref: 167. Journal code: 8102988. ISSN: 0250-7005. Pub. country: Greece. Language: English.

AB Cross-resistance between different cytostatic agents which are structurally and functionally dissimilar is a common phenomenon called multidrug resistance (MDR). The best characterized mechanism of MDR involves **P-glycoprotein.** However, this does not completely explain MDR. Within the last few years, two new genes that can confer MDR have been identified (MRP and LRP). Furthermore, topoisomerase II has been associated with a special form of MDR. During the past several years, considerable interest has been shown in strategies to reverse MDR by using pharmacological compounds, monoclonal antibodies, immunotoxins, **bispecific antibodies**, antisense oligodeoxynucleotides, ribozymes, and albumin-conjugated drugs in in vitro and in vivo assays. All these experimental assays demonstrated that MDR can be circumvented. Two agents that have received the most attention in the clinic are verapamil and cyclosporin A. Despite some promising results (especially in hematological malignancies), the results obtained in the treatment of solid **tumors** with modulators have so far been quite disappointing. This may be explained by the fact that the MDR phenotype alone does not completely account for the resistance of human cancer. Several other resistance-related proteins (e.g., glutathione S-transferase, metallothionein, O6-alkylguanine-DNA-alkyltransferase, thymidylate synthase, dihydrofolate reductase, heat shock proteins) can be also expressed in resistant **tumors.** Additionally, cell proliferation, vascularization and apoptosis are involved in resistance.

L9 ANSWER 5 OF 10 MEDLINE on STN

97300052. PubMed ID: 9155164. Overcoming of multidrug resistance by anti-**P-glycoprotein** monoclonal antibody. Nokihara H; Yano S; Sone S. (Third Department of Internal Medicine, University of Tokushima School of Medicine.) Nippon rinsho. Japanese journal of clinical medicine, (1997 May) 55 (5) 1128-34. Ref: 22. Journal code: 0420546. ISSN: 0047-1852. Pub. country: Japan. Language: Japanese.

AB **P-glycoprotein** is one of the key molecules in multidrug resistance. Monoclonal antibodies against **P-glycoprotein** could be useful tools for killing MDR **tumor** cells. To overcome multidrug resistance, many anti-**P-glycoprotein** monoclonal antibodies have been made such as MRK16 and MRK17. Conjugated moAb, such as **bispecific antibody**, immunotoxin and radioisotope conjugates have also been constructed to enhance the anti-**tumor** activity of moAb. Cytokine-gene transduction for accumulation and activation of monocytes may be hopeful to augment the therapeutic efficiency of anti-**P-glycoprotein** antibody. For clinical use, construction of human moAb, overcoming **tumor** heterogeneity, and protection of normal tissue by specifically targeting the **tumor** cells should be essential.

L9 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN

1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US9491 19950801. PRIORITY: US 1994-286430 19940805.

AB Polyspecific immunoconjugates and antibody composites that bind a multidrug transporter protein and an antigen associated with a **tumor** or infectious agent are used to overcom the multidrug resistant phenotype. These immunoconjugates and composites also can be used diagnostically to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant **tumor** cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents. In example, polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin, dextran and anti-**P-glycoprotein**/anti-carcinoembryonic antigen **bispecific antibody** were prepared for colon cancer treatment. 99mTc- and 111Indium-labeled polyspecific immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa were also prepared

L9 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
1992:529855 Document No. 117:129855 **Bispecific antibodies** to a **tumor** cell and to a cytotoxic cell, method of production, and uses thereof. Ring, David; Shi, Tian Xiang (Cetus Corp., USA). PCT Int. Appl. WO 9208802 A1 19920529, 61 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US6949 19910924. PRIORITY: US 1990-605399 19901029.

AB A **bispecific antibody** is made by a hybrid hybridoma and is capable of binding to a multiple drug-resistant **tumor** cell and to a cytotoxic cell. Cells of hybridoma 3G8 secreting a mouse monoclonal antibody to human Fcγ receptor III, i.e. FcγRIII or IgG receptors on cytotoxic cells, were stained with rhodamine 123 and then fused at 1:20 with hybridoma 15D3 cells secreting monoclonal antibodies to multi-drug resistance antigen, **P-glycoprotein.** **Bispecific antibodies** 34.5 and 41.6 mediated lysis of the drug-resistant K562/R7 cells at .apprx.2 times background.

L9 ANSWER 8 OF 10 MEDLINE on STN
94076869. PubMed ID: 1285219. Antibody-directed therapy of multidrug-resistant **tumor** cells. Efferth T; Volm M. (German Cancer Centre, Heidelberg.) Medical oncology and tumor pharmacotherapy, (1992) 9 (1) 11-9. Ref: 43. Journal code: 8405039. ISSN: 0736-0118. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The major obstacle to effective cancer chemotherapy is the resistance of **tumor** cells to cytostatic agents. **Tumor** cells frequently express a multidrug-resistance (MDR) phenotype. In an effort to devise new strategies to overcome MDR, antibody-directed approaches for the eradication of MDR cells have been developed. Experimental data have shown that multidrug-resistant **tumor** cells can be efficiently killed by monoclonal antibodies, immunotoxins, or **bispecific antibodies.** The current experimental results indicate that an antibody-directed therapeutic approach to eradication of MDR cells might be a promising adjunct to conventional chemotherapy of cancer patients.

L9 ANSWER 9 OF 10 MEDLINE on STN
91349601. PubMed ID: 1715368. Selection of hybrid hybridomas by flow cytometry using a new combination of fluorescent vital stains. Shi T; Eaton A M; Ring D B. (Department of Immunology, Cetus Corporation, Emeryville, CA 94608.) Journal of immunological methods, (1991 Aug 9) 141 (2) 165-75. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB A new combination of fluorescent dyes (rhodamine 123 and hydroethidine) was used to internally label hybridoma fusion partners. Murine hybridoma 520C9 (recognizing human c-erbB-2) was labeled with hydroethidine. Murine hybridoma 3G8 (recognizing human Fc gamma receptor III) was labeled with rhodamine 123, and verapamil was used to block rhodamine efflux via **P-glycoprotein.** Viability assays showed little cytotoxicity from these dyes at the concentrations used. The labeled cells were fused with polyethylene glycol, sorted for dual fluorescence on an Epics V cell sorter, and cloned. Hybrid hybridomas producing **bispecific antibodies** were selected for ability to promote lysis of SK-Br-3 breast cancer cells by human mononuclear cells. Several positive clones were obtained and shown to have a double content of DNA. **Bispecific antibody** produced by subclone 2B1 was purified by anion exchange chromatography and shown to bind both **tumor** cells and Fc gamma R III bearing cells. Using two parameter flow cytometric analysis, we were able to measure a 'bridging' effect of this **bispecific antibody**, which caused formation of complexes between PMNs and SK-Br-3 cells. Either parental antibody could compete with **bispecific antibody** to block such complexing. This fusion method provides several advantages over other techniques presently used (speed, convenience, low toxicity and automatic exclusion of dead cells) and can be applied to produce other hybrid hybridomas.

L9 ANSWER 10 OF 10 MEDLINE on STN

90007768. PubMed ID: 2793246. **Bispecific antibodies** reactive with the multidrug-resistance-related glycoprotein and CD3 induce lysis of multidrug-resistant **tumor** cells. Van Dijk J; Tsuruo T; Segal D M; Bolhuis R L; Colognola R; van de Griend R J; Fleuren G J; Warnaar S O. (Department of Pathology, State University Leiden, The Netherlands.) International journal of cancer. Journal international du cancer, (1989 Oct 15) 44 (4) 738-43. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB We describe the lysis of multidrug-resistant (MDR) **tumor** cells by various lymphocytic effector cells, retargeted with **bispecific antibodies** (heteroconjugates). The Ab-heteroconjugate used was prepared by chemically cross-linking the OKT3 monoclonal antibody (MAb) reactive with CD3 antigen on T lymphocytes, with the MRK16 MAb, which recognizes the MDR-associated **P-glycoprotein.** Cloned TCR alpha beta/CD3+ T lymphocytes, OKT3-activated peripheral-blood mononuclear cells and peripheral-blood mononuclear blood lymphocytes, stimulated with allogeneic irradiated cells in a mixed lymphocyte culture, could be induced to lyse MDR ovarian **tumor** cells in the presence of Ab-heteroconjugate CD3/MRK16, whereas the drug-sensitive parental **tumor** cells lacking the **P-glycoprotein** were not lysed by these retargeted effector cells. Cloned TCR gamma delta/CD3+ T lymphocytes showed a high MHC-unrestricted lysis of MDR **tumor** cells. Addition of Ab-heteroconjugate CD3/MRK16 could therefore not enhance target-cell lysis. Melanoma **tumor** cells transfected with the mdr-I gene which codes for the **P-glycoprotein** were also efficiently lysed by Ab-heteroconjugate retargeted cloned TCR alpha beta/CD3+ T cells. **Tumor** cell lines derived from organs known to express the **P-glycoprotein** also were lysable by the retargeted effector cells.

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=> s l1 and OtrB
L10          2 L1 AND OTRB

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PROCESSING COMPLETED FOR L10
L11          2 DUP REMOVE L10 (0 DUPLICATES REMOVED)

=> d l11 cbib abs 1-2
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L11 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN

2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

```
AB   Immunoconjugates of a diagnostic agent and an antibody component that
     binds an epitope of a multidrug transporter protein are disclosed.  Thesse
     immunoconjugates are used in in vivo diagnostic methods to determine whether
     the failure of traditional chemotherapy is due to the presence of
     multidrug resistant tumor cells, multidrug resistant HIV-infected cells or
     multidrug resistant infectious agents.

L11  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a tumor or
     infectious agent are used to overcom the multidrug resistant phenotype.
     These immunoconjugates and composites also can be used diagnostically to
     determine whether the failure of traditional chemotherapy is due to the
     presence of multidrug resistant tumor cells, multidrug resistant
     HIV-infected cells or multidrug resistant infectious agents.  In example,
     polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin,
     dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer
     treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
     targeted to multidrug resistant Pseudomonas aeruginosa were also prepared


=>

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L12           2 L1 AND "TEL(L)"

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PROCESSING COMPLETED FOR L12
L13            2 DUP REMOVE L12 (0 DUPLICATES REMOVED)

=> d l13 1-2 cbib abs

L13  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug resistance in
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     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
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     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
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     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
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     multidrug resistant tumor cells, multidrug resistant HIV-infected cells or
     multidrug resistant infectious agents.

L13  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
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     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
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     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
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     bispecific antibody were prepared for colon cancer
     treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
     targeted to multidrug resistant Pseudomonas aeruginosa were also prepared


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L14           2 L1 AND "MMR"

=> dup remove l14
PROCESSING COMPLETED FOR L14
L15           2 DUP REMOVE L14 (0 DUPLICATES REMOVED)

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L15  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017  Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
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     multidrug resistant infectious agents.

L15  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658  Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
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     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
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     treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
     targeted to multidrug resistant Pseudomonas aeruginosa were also prepared


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L16          2 L1 AND "ACTII"

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PROCESSING COMPLETED FOR L16
L17          2 DUP REMOVE L16 (0 DUPLICATES REMOVED)

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L17  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017  Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
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     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
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L17  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658  Document No. 124:315061  Polyspecific immunoconjugates and
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     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
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     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
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     bispecific antibody were prepared for colon cancer
     treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
     targeted to multidrug resistant Pseudomonas aeruginosa were also prepared
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L18        3891 (GOLDENBERG D?/AU)

=> s l18 and polyspecific antibod?
L19           1 L18 AND POLYSPECIFIC ANTIBOD?

=> d l19 cbib abs

L19  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:880995   Document No. 134:41104  Immunotherapy of autoimmune disorders
     using antibodies which target B-cells.  Goldenberg, David M.;
     Hansen, Hans J. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000074718
     A1 20001214, 39 pp.  DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ,
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     YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF,
     CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML,
     MR, NE, NL, PT, SE, SN, TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION:
     WO 2000-US15780 20000609.  PRIORITY: US 1999-PV138284 19990609.
AB   Antibodies that bind with a B-cell antigen provide an effective means to
     treat autoimmune disorders.  Antibodies and fragments, which may be
     conjugated or naked, are used alone or in multimodal therapies.  The
     antibodies may be bispecific antibodies which may be produced
     recombinantly as fusion proteins, or as hybrid, polyspecific
     antibodies.


=> s l18 and bispecific antibod?
L20         103 L18 AND BISPECIFIC ANTIBOD?

=> s l20 and multidrug resistance or "MDR"
L21       25690 L20 AND MULTIDRUG RESISTANCE OR "MDR"

=> s l20 and multidrug resistance
L22           2 L20 AND MULTIDRUG RESISTANCE

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PROCESSING COMPLETED FOR L22
L23           2 DUP REMOVE L22 (0 DUPLICATES REMOVED)

=> d l23 1-2 cbib abs

L23  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug
     resistance in cancer and infectious lesions using
     immunoconjugates.  Goldenberg, David M. (Immunomedics, Inc.,
     USA).  PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp.  DESIGNATED
     STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR,
     CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP,
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     APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US 1998-PV99304
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L23  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO
     9604313 A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG,
     BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG,
     KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO,
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     CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE,
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     bispecific antibody were prepared for colon cancer
     treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
     targeted to multidrug resistant Pseudomonas aeruginosa were also prepared


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PROCESSING IS APPROXIMATELY  35% COMPLETE FOR L18
PROCESSING IS APPROXIMATELY  88% COMPLETE FOR L18
PROCESSING COMPLETED FOR L18
L24        1816 DUP REMOVE L18 (2075 DUPLICATES REMOVED)

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PROCESSING COMPLETED FOR L20
L25          62 DUP REMOVE L20 (41 DUPLICATES REMOVED)

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L25  ANSWER 1 OF 62     MEDLINE on STN                  DUPLICATE 1
2004262654.    PubMed ID: 15161715.   Improved targeting of pancreatic cancer:
     experimental studies of a new bispecific antibody,
     pretargeting enhancement system for immunoscintigraphy. Cardillo Thomas M;
     Karacay Habibe; Goldenberg David M; Yeldell Dion; Chang
     Chien-Hsing; Modrak David E; Sharkey Robert M; Gold David V. (Garden State
     Cancer Center, Belleville, New Jersey 07109, USA. ) Clinical cancer
     research : an official journal of the American Association for Cancer
     Research, (2004 May 15) 10 (10) 3552-61.  Journal code: 9502500. ISSN:
     1078-0432. Pub. country: United States. Language: English.
AB   PURPOSE: The early detection and diagnosis of pancreatic cancer remains a
     major clinical challenge in which imaging procedures have a central role.
     The purpose of this study was to evaluate a pretargeting method with a
     bispecific PAM4 (bsPAM4; anti-MUC1) antibody for radioimmunoscintigraphy
     of experimental human pancreatic cancer.  EXPERIMENTAL DESIGN: A
     bispecific F(ab')(2) antibody was generated from chimeric PAM4 Fab' and
     murine 734 (anti-indium-diethylenetriaminepentaacetic acid) Fab' fragments
     and then used in conjunction with 2 peptide haptens ((111)In-IMP-156 and
     (99m)Tc-IMP-192).  Biodistribution studies and radioimmunoscintigraphic
     imaging properties of the radiolabeled bsPAM4, and pretargeted,
     radiolabeled peptides were examined in the CaPan1 human pancreatic tumor
     grown as s.c. xenografts in athymic nude mice.  Tumor uptake and
     tumor:nontumor ratios were compared with a nontargeting irrelevant
     anti-CD20, bispecific rituximab, radiolabeled peptides alone, and with
     directly labeled PAM4.  RESULTS: Biodistribution results indicated
     significantly greater tumor uptake of radiolabeled peptides at 3 h after
     injection when pretargeting was performed with bsPAM4 as compared with the
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bispecific rituximab [20.2 +/- 5.5 percentage of injected dose per gram of tissue (%ID/g) versus 0.9 +/- 0.1%ID/g, respectively, for (111)In-IMP-156, and 16.8 +/- 4.8%ID/g versus 1.1 +/- 0.2%ID/g, respectively, for (99m)Tc-IMP-192]. Similar results were obtained at the 24-h time point. Tumor:nontumor ratios were >30 for all of the tissues except the kidneys, where a ratio of 7.8 +/- 2.8 was observed. By immunoscintigraphy, tumors could be visualized as early as 30 min after injection of the radiolabeled peptide. CONCLUSIONS: These studies demonstrate the feasibility of using the pretargeted, **bispecific antibody** technology for nuclear imaging of pancreatic cancer. The advantage of pretargeted bsPAM4 antibody as an imaging platform is the high specificity for pancreatic cancer as compared with the physicochemical parameters identified by current imaging technologies.

L25 ANSWER 2 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:957005 The Genuine Article (R) Number: 847UG. Studies to determine the most optimal form of the **bispecific antibody** anti-RCC x anti-DTIn-1 for pretargeting: IgG vs. F(ab ')(2)-fragments. van Schaijk F G (Reprint); Oosterwijk E; McBride W J; **Goldenberg D M**; Corstens F H M; Boerman O C. UMCN St Radboud, Nijmegen, Netherlands; Immunomed, Morris Plains, NJ USA. EUROPEAN JOURNAL OF NUCLEAR MEDICINE AND MOLECULAR IMAGING (AUG 2004) Vol. 31, Supp. [2], pp. S280-S280. Publisher: SPRINGER. 233 SPRING STREET, NEW YORK, NY 10013 USA. ISSN: 1619-7070. Pub. country: Netherlands; USA. Language: English.

L25 ANSWER 3 OF 62 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2004483556 EMBASE Advancing role of radiolabeled antibodies in the therapy of cancer. **Goldenberg D.M.**. Dr. D.M. Goldenberg, Garden State Cancer Center, Ctr. for Molec. Med. and Immunology, Belleville, NJ 07109, United States. dmg.gscancer@att.net. Journal of Clinical Ligand Assay 27/1 (40-55) 2004.
Refs: 139.
ISSN: 1081-1672. CODEN: JCLAF5. Pub. Country: United States. Language: English. Summary Language: English.

AB This review focuses on the use of radiolabeled antibodies in the therapy of cancer, termed radioimmunotherapy (RAIT). Basic problems concerned with the choice of antibody, radionuclide, and physiology of the tumor and host are discussed, followed by a review of the pertinent clinical publications of various radioantibody constructs in the treatment of hematopoietic and solid tumors of diverse histopathology, grade, and stage, and in different clinical settings. Factors such as dose rate delivered, tumor size, and radiosensitivity play a major role in determining therapeutic response, while target-to-nontarget ratios and, particularly, circulating radioactivity to the bone marrow determine the principal dose-limiting toxicities. RAIT appears to be gaining a place in the therapy of hematopoietic neoplasms, such as non-Hodgkin's lymphoma: several agents are advancing in clinical trials toward registration, and one has recently been approved by the FDA. Although RAIT of solid tumors has shown less progress, use of pretargeting strategies, such as an afinity-enhancement system consisting of **bispecific antibodies** separating targeting from delivery of the radiotherapeutic, appears to enhance tumor-to-nontumor ratios, and may increase radiation doses to tumors more selectively than directly labeled antibodies.

L25 ANSWER 4 OF 62 MEDLINE on STN DUPLICATE 2

2004034992. PubMed ID: 14734668. Reagents and methods for PET using **bispecific antibody** pretargeting and 68Ga-radiolabeled bivalent hapten-peptide-chelate conjugates. Griffiths Gary L; Chang Chien-Hsing; McBride William J; Rossi Edmund A; Sheerin Agatha; Tejada German R; Karacay Habibe; Sharkey Robert M; Horak Ivan D; Hansen Hans J; **Goldenberg David M.** (Immunomedics, Inc., Morris Plains, New Jersey. Garden State Cancer Center, Belleville, New Jersey 07950, USA.. ggriffiths@immunomedics.com) . Journal of nuclear medicine : official publication, Society of Nuclear Medicine, (2004 Jan) 45 (1) 30-9. Journal code: 0217410. ISSN: 0161-5505. Pub. country: United States. Language: English.

AB The aim of this work was to develop reagents and methods potentially useful in PET, using (68)Ga in a 2-step pretargeting protocol. METHODS: We prepared **bispecific antibodies** (bsAbs) for disease-specific targeting of carcinoembryonic antigen-positive cells and recognition of later-administered bivalent hapten-peptide conjugates. The secondary antibody arm (antibody 679) recognizes a histaminyl-succinyl-glycine (HSG) structural subunit. The bsAbs were prepared as Fab' x Fab' conjugates using chemical cross-linking methods and as bispecific diabodies using recombinant DNA technologies. A HSG-bivalent hapten conjugate bearing the macrocyclic ring chelating agent 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA) was designed to be readily radiolabeled with (68)Ga taken directly from a (68)Ge/(68)Ga generator system. Reagents were tested in vitro and, then, for their targeting properties in a preclinical animal model of human cancer. RESULTS: A chemically cross-linked hMN-14 x 679 F(ab')(2) and a fully humanized bispecific diabody construct (BS1.5H), expressed in Escherichia coli, were prepared for this work. We synthesized the bivalent peptide termed IMP 241 [DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH(2)] and labeled it with (68)Ga and (67)Ga at temperatures from 45 degrees C to 100 degrees C, over times of 15 min to 1 h, establishing 15 min at 95 degrees C as a useful condition for (68)Ga labeling. When we formulated the IMP 241 bivalent hapten-peptide with ammonium acetate buffer at pH 4-5 and eluted the (68)Ga from the generator directly into the peptide solution, we achieved an almost quantitative incorporation of the (68)Ga into IMP 241, as analyzed by size-exclusion high-performance liquid chromatography, after mixing the complex with the 679 antibody. For in vivo studies we used (67)Ga-IMP 241 as a surrogate for (68)Ga-IMP 241, in view of the short, 68-min half-life of the (68)Ga nuclide. The (67)Ga-IMP 241 was successfully pretargeted to human colon tumor xenografts in athymic mice with both the chemical and the diabody bispecific proteins. High tumor-to-normal tissue ratios for (67)Ga uptake were found for all tissues at 1 to 6 h after injection of (67)Ga-IMP 241. When using the BS1.5H diabody for pretargeting, tumor-to-blood, tumor-to-liver, and tumor-to-lung ratios of (67)Ga-IMP 241 at 1 and 3 h after injection were 41:1 and 137:1, 51:1 and 106:1, and 16:1 and 46:1, respectively. CONCLUSION: The general approach described, along with the new compositions and the labeling methods we have developed, may eventually allow for use of (68)Ga-labeled specific targeting agents in a routine clinical PET application.

L25 ANSWER 5 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN

2003:971926 Document No. 140:31491 Methods and compositions for intravesical therapy of bladder cancer. Griffiths, Gary L.; **Goldenberg, David M.** (Immunomedics, Inc., USA; McCall, John Douglas). PCT Int. Appl. WO 2003101496 A1 20031211, 45 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-GB2387 20030602. PRIORITY: US 2002-PV384391 20020603.

AB Disclosed is a method for treating bladder cancer by administering via the urethra a multispecific antibody comprising at least one targeting arm that binds a bladder cancer antigen and at least one capture arm that binds a carrier conjugated to one or more therapeutic agents, allowing said multispecific antibody to localize at the site of said bladder cancer, allowing any free multispecific antibody to substantially clear from the patient; and (b) administering a therapeutically effective amount of the carrier conjugated to one or more therapeutic agents. A patient with a superficial cancer of the urinary bladder is treated with a 1:1 M mixture of a **bispecific antibody** hMN-14-Fab'-linker-Fab'-679 prepared from monoclonal antibody hMN-14 [humanized anticarcinoembryonic antigen(CEA)] and anti-hapten antibody termed 679 [murine antihistaminyl-glycyl-succinimidyl(HSG) moiety] and Y-90 radiolabeled bivalent hapten peptide Y-90-IMP 241 introduced into the bladder via a urethral catheter.

L25 ANSWER 6 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
2003:971925 Document No. 140:31451 Methods and compositions for radioimmunotherapy of brain and central nervous system tumors. Griffiths, Gary L.; **Goldenberg, David M.** (Immunomedics, Inc., USA; McCall, John Douglas). PCT Int. Appl. WO 2003101495 A1 20031211, 43 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-GB2334 20030528. PRIORITY: US 2002-PV383617 20020529.

AB A method for treating a brain tumor by administering to a patient (i) a multispecific antibody comprising at least one targeting arm that binds to a brain cancer antigen and at least one capture arm that binds to a radionuclide carrier; (ii) a therapeutic radionuclide carrier to which is bound a therapeutic radionuclide, wherein the multispecific antibody, radionuclide carrier and therapeutic radionuclide are administered in a substantially equimolar ratio. Several examples are provided on the preparation of **bispecific antibody** (anti-tenascin and anti-hapten 679), the preparation of radiolabeled bivalent haptens, and the treatment of brain cancer patients with the above immunoradiotherapy regimen.

L25 ANSWER 7 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
2003:719520 Document No. 139:259964 **Bispecific antibody** mutants with enhanced rate of clearance for diagnosis and treatment of immune, autoimmune, cardiovascular and neurological diseases. Qu, Zhengxing; Hansen, Hans; **Goldenberg, David M.** (Immunomedics, Inc., USA; McCall, John Douglas). PCT Int. Appl. WO 2003074569 A2 20030912, 68 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-GB871 20030303. PRIORITY: US 2002-PV361037 20020301.

AB A mutant **bispecific antibody** that includes (a) a human hinge constant region from IgG having one or more amino acid mutations in the CH2 domain, (b) two scFvs; and (c) two Fvs has been constructed. This type of antibody displays enhanced clearance, which has been found to be particularly useful in the context of pre-targeting methods. The **bispecific antibody** mutants are useful for conjugated with therapeutic or diagnostic agents for diagnosis and treatment of immune disease, autoimmune disease, cardiovascular disease, neurol. disease, organ graft rejection, or graft vs. host disease.

L25 ANSWER 8 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
2003:836381 Document No. 139:341719 Use of bi-specific antibodies for pre-targeting diagnosis and therapy. **Goldenberg, David M.**; Hansen, Hans J.; Leung, Shui-on; McBride, William J.; Qu, Zhengxing (Immunomedics, Inc., USA). U.S. Pat. Appl. Publ. US 2003198595 A1 20031023, 59 pp., Cont.-in-part of U.S. Ser. No. 823,746. (English).

CODEN: USXXCO. APPLICATION: US 2002-150654 20020517. PRIORITY: US 1998-PV90142 19980622; US 1998-PV104156 19981014; US 1999-382186 19990823; US 2001-823746 20010403.

AB The present invention relates to a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable construct. The targetable construct comprises a carrier portion which comprises or bears at least one epitope recognizable by at least one arm of said bi-specific antibody or antibody fragment. The targetable construct further comprises one or more therapeutic or diagnostic agents or enzymes. The invention provides constructs and methods for producing the bi-specific antibodies or antibody fragments, as well as methods for using them.

L25 ANSWER 9 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:51012 The Genuine Article (R) Number: 756LU. Recombinant **bispecific antibodies** (bsMAb) for improved pretargeted therapy of CEA-expressing tumors.. Sharkey R M (Reprint); Karacay H; Rossi E A; Chang C H; McBride W J; Horak I D; Hansen H J; **Goldenberg D M.** Ctr Mol Med & Immunol, Belleville, NJ USA; IBC Pharmaceut Inc, Morris Plains, NJ USA; Immunomed Inc, Morris Plains, NJ USA. CLINICAL CANCER RESEARCH (1 DEC 2003) Vol. 9, No. 16, Part 2, Supp. [S], pp. 6193S-6193S. Publisher: AMER ASSOC CANCER RESEARCH. 615 CHESTNUT ST, 17TH FLOOR, PHILADELPHIA, PA 19106-4404 USA. ISSN: 1078-0432. Pub. country: USA . Language: English.

L25 ANSWER 10 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:50993 The Genuine Article (R) Number: 756LU. **Bispecific antibody** (bsMAb) pretargeting for the radioimmunotherapy (RAIT) of colorectal cancer using a bsMAb against colon-specific antigen-p (CSAp).. Karacay H (Reprint); Sharkey R M; Newsome G; Yeldell D; McBride W J; Chang C H; Horak I D; Hansen H J; **Goldenberg D M.** Ctr Mol Med & Immunol, Belleville, NJ USA; Immunomed Inc, Morris Plains, NJ USA; IBC Pharmaceut Inc, Morris Plains, NJ USA. CLINICAL CANCER RESEARCH (1 DEC 2003) Vol. 9, No. 16, Part 2, Supp. [S], pp. 6188S-6188S. Publisher: AMER ASSOC CANCER RESEARCH. 615 CHESTNUT ST, 17TH FLOOR, PHILADELPHIA, PA 19106-4404 USA. ISSN: 1078-0432. Pub. country: USA. Language: English.

L25 ANSWER 11 OF 62 MEDLINE on STN DUPLICATE 3

2003445639. PubMed ID: 14506196. Pharmacokinetics and dosimetry studies for optimization of anti-carcinoembryonic antigen x anti-hapten **bispecific antibody**-mediated pretargeting of Iodine-131-labeled hapten in a phase I radioimmunotherapy trial. Kraeber-Bodere Francoise; Faivre-Chauvet Alain; Ferrer Ludovic; Vuillez Jean-Philippe; Brard Pierre-Yves; Rousseau Caroline; Resche Isabelle; Devillers Anne; Laffont Sophie; Bardies Manuel; Chang Ken; Sharkey Robert M; **Goldenberg David M**; Chatal Jean-Francois; Barbet Jacques. (Nuclear Medicine Department, Rene Gaudducheau Cancer Center, 44805 Saint Herblain, France.) Clinical cancer research : an official journal of the American Association for Cancer Research, (2003 Sep 1) 9 (10 Pt 2) 3973S-81S. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB PURPOSE: Pharmacokinetics and dosimetry of hMN-14 x m734 bispecific monoclonal antibody (BsMAb) and (131)I-labeled di-diethylenetriaminepentaacetic acid-indium ((131)I-hapten) were studied to optimize pretargeted radioimmunotherapy. EXPERIMENTAL DESIGN: Thirty-five patients with carcinoembryonic antigen-expressing tumors were included. In a first group of 12 patients, (131)I-trace-labeled BsMAb doses were escalated from 10 to 100 mg/m(2), and 3.7 GBq of (131)I-hapten were administered 7 days later. In a second group, 12 patients received 75 mg/m(2) BsMAb and 2.6-4.2 GBq of (131)I-hapten 5 days later. The BsMAb dose was then reduced to 40 mg/m(2), and 10 patients received 1.9-5.5 GBq of (131)I-hapten. Blood samples were collected. Biodistribution was monitored by quantitative scintigraphy. RESULTS: Directly labeled BsMAb pharmacokinetics was described by two exponentials: half-lives were 8.1 h (2.0-18.1 h) and 48.2 h (22.8-79.4 h); blood clearance was 123 ml/h (64-195 ml/h). With a 7-day interval, 10 or 30 mg/m(2) BsMAb resulted in fast elimination and very low tumor uptake of hapten, whereas 50 or 100 mg/m(2) resulted in favorable tumor accretion. With 75 mg/m(2) BsMAb and a 5-day interval, hapten clearance was 152 ml/h (81-298 ml/h). Calculated radiation dose to tumor was 3.9 Gy/GBq (0.4-22.4 Gy/GBq) for the hapten, compared with 2.0 Gy/GBq (0.3-3.8 Gy/GBq) for the BsMAb, but hematological toxicity prevented dose escalation. Reduction of the BsMAb dose to 40 mg/m(2) accelerated hapten clearance to 492 ml/h (113-2544 ml/h) and reduced hematological toxicity without compromising tumor uptake [5.2 Gy/GBq (0.5-12.6 Gy/GBq)]. CONCLUSIONS: Optimized BsMAb doses and time interval will allow for the administration of higher, tumoricidal, activity doses.

L25 ANSWER 12 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
2003:847738 Document No. 140:195455 Pharmacokinetics and dosimetry studies for optimization of anti-carcinoembryonic antigen x anti-hapten **bispecific antibody**-mediated pretargeting of iodine-131-labeled hapten in a phase I radioimmunotherapy trial. Kraeber-Bodere, Francoise; Faivre-Chauvet, Alain; Ferrer, Ludovic; Vuillez, Jean-Philippe; Brard, Pierre-Yves; Rousseau, Caroline; Resche, Isabelle; Devillers, Anne; Laffont, Sophie; Bardies, Manuel; Chang, Ken; Sharkey, Robert M.; **Goldenberg, David M.**; Chatal, Jean-Francois; Barbet, Jacques (Nuclear Medicine Department, Rene Gauducheau Cancer Center, Saint Herblain, F-44805, Fr.). Clinical Cancer Research, 9(10, Pt. 2), 3973s-3981s (English) 2003. CODEN: CCREF4. ISSN: 1078-0432. Publisher: American Association for Cancer Research.

AB Purpose: Pharmacokinetics and dosimetry of hMN-14 + m734 bispecific monoclonal antibody (BsMAb) and 131I-labeled di-diethylenetriaminepentaacetic acid-indium (131I-hapten) were studied to optimize pretargeted radioimmunotherapy. Exptl. Design: Thirty-rive patients with carcinoembryonic antigen-expressing tumors were included. In a first group of 12 patients, 131I-trace-labeled BsMAb doses were escalated from 10 to 100 mg/m2, and 3.7 GBq of 131I-hapten were administered 7 days later. In a second group, 12 patients received 75 mg/m2 BsMAb and 2.6-4.2 GBq of 131I-hapten 5 days later. The BsMAb dose was then reduced to 40 mg/m2, and 10 patients received 1.9-5.5 GBq of 131I-hapten. Blood samples were collected. Biodistribution was monitored by quant. scintigraphy. Results: Directly labeled BsMAb pharmacokinetics was described by two exponentials: half-lives were 8.1 h (2.0-18.1 h) and 48.2 h (22.8-79.4 h); blood clearance was 123 mL/h (64-195 mL/h). With a 7-day interval, 10 or 30 mg/m2 BsMAb resulted in fast elimination and very low tumor uptake of hapten, whereas 50 or 100 mg/m2 resulted in favorable tumor accretion. With 75 mg/m2 BsMAb and a 5-day interval, hapten clearance was 152 mL/h (81-298 mL/h). Calculated radiation dose to tumor was 3.9 Gy/GBq (0.4-22.4 Gy/GBq) for the hapten, compared with 2.0 Gy/GBq (0.3-3.8 Gy/GBq) for the BsMAb, but hematol. toxicity prevented dose escalation. Reduction of the BsMAb dose to 40 mg/m2 accelerated hapten clearance to 492 mL/h (113-2544 mL/h) and reduced hematol. toxicity without compromising tumor uptake [5.2 Gy/GBq (0.5-12.6 Gy/GBq)]. Conclusions: Optimized BsMAb doses and time interval will allow for the administration of higher, tumoricidal, activity doses.

L25 ANSWER 13 OF 62 MEDLINE on STN DUPLICATE 4
2003445632. PubMed ID: 14506188. Optimizing **bispecific antibody** pretargeting for use in radioimmunotherapy. Sharkey Robert M; Karacay Habibe; Richel Heidi; McBride William J; Rossi Edmund A; Chang Ken; Yeldell Dion; Griffiths Gary L; Hansen Hans J; **Goldenberg David M.** (Center for Molecular Medicine and Immunology, Belleville, New Jersey 07109, USA.. rmsharkey@gscancer.org) . Clinical cancer research : an official journal of the American Association for Cancer Research, (2003 Sep 1) 9 (10 Pt 2) 3897S-913S. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB PURPOSE: With increasing interest in pretargeting procedures for improving the delivery of radionuclides for cancer imaging and therapy, this investigation was undertaken to examine how to optimize a bispecific monoclonal antibody (bsMAb) pretargeting procedure for therapeutic applications. EXPERIMENTAL DESIGN: The model system examined was a bsMAb composed of two Fab' fragments, one from a humanized anti-carcinoembryonic antigen antibody (hMN-14), and the other a murine antibody (679) against histamine-succinyl-glycine. These Fab' fragments were chemically conjugated to form a F(ab')(2) that is joined by a stable thioether bond. The peptide used for these studies (IMP-241) contained two histamine-succinyl-glycine moieties for binding to the 679 portion of the bsMAb and a single 1,4,7,10-tetra-azacyclododecane N,N',N",N"'-tetraacetic acid chelate for radiolabeling with (111)In. RESULTS: The bsMAb cleared rapidly in nude mice bearing the GW-39 human colonic cancer xenograft. Administration of a radiolabeled peptide 1 day after the bsMAb, using a bsMAb/peptide mole injection ratio of 10:1, allowed for higher tumor accretion than if delayed by 2 days. Tumor uptake measured 3 h after the peptide injection given 1 day after the bsMAb was 11.3 +/- 2.2% percentage of injected dose/gram (%ID/g), with just 2.9 +/- 0.4% ID/g of the bsMAb in the tumor at this time. Tumor/blood ratios were 8.1 +/- 2.1. Peptide uptake was highest in the kidneys, but even so, the tumor/kidney ratio was 2.5 +/- 1.9 just 3 h after the peptide injection. Although low bsMAb/peptide mole injection ratios allow for greater concentrations of the peptide in the tumor, kidney uptake is increased at a proportionally higher amount than in the tumor. Therefore, a bsMAb/peptide injection ratio of 10:1 with a 24-h interval was preferred for pretargeting. Increasing the bsMAb dose, and thereby increasing the bsMAb/peptide injection ratio, further enhanced the delivery of the radiolabeled peptide to the tumor, but the interval spacing between the bsMAb and peptide had to be increased. Despite having a lower %ID/g of the bsMAb in the tumor, with a bsMAb/peptide injection ratio of 50:1 and a 48-h interval, tumor uptake of the (111)In-peptide was nearly 30% ID/g, a 1.6-fold improvement over that seen with the 10:1/24-h interval pretargeting group, and tumor/blood was 35:1, and tumor/kidney ratio was 8:1. Two fractionation strategies were also examined. Giving two equal fractions of peptide after a single injection of bsMAb loaded more moles of peptide into the tumor but would not permit higher radioactivity delivery than what could be achieved with a single injection. However, area under the curve analysis indicated that giving repeated cycles of the bsMAb followed by the peptide would enable improvements in the amount of radioactivity delivered to the tumor without increasing the amount delivered to normal tissues, but the timing of the bsMAb/peptide cycles was important to optimize this process. Finally, it was noted that larger tumors (e.g. those > 0.3 g) were more likely to have higher peptide uptake in a pretargeting procedure than smaller tumors (e.g., those of approximately 0.1 g), perhaps due to the greater mass of the bsMAb localized in the larger tumors, but also possibly because of better blood supply in these tumors. CONCLUSIONS: These studies reveal principles that might be applied generally to other pretargeting procedures and demonstrate how a bsMAb pretargeting method could potentially exceed a directly radiolabeled antibody in its ability to deliver radionuclides for cancer therapy.

L25 ANSWER 14 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN

2003:847730 Document No. 140:419948 Optimizing **bispecific antibody** pretargeting for use in radioimmunotherapy. Sharkey, Robert M.; Karacay, Habibe; Richel, Heidi; McBride, William J.; Rossi, Edmund A.; Chang, Ken; Yeldell, Dion; Griffiths, Gary L.; Hansen, Hans J.; **Goldenberg, David M.** (Center for Molecular Medicine and Immunology, Belleville, NJ, 07109, USA). Clinical Cancer Research, 9(10, Pt. 2), 3897s-3913s (English) 2003. CODEN: CCREF4. ISSN: 1078-0432. Publisher: American Association for Cancer Research.

AB Purpose: With increasing interest in pretargeting procedures for improving the delivery of radionuclides for cancer imaging and therapy, this investigation was undertaken to examine how to optimize a bi-specific monoclonal antibody (bsMAb) pretargeting procedure for therapeutic applications. Exptl. Design: The model system examined was a bsMAb composed of two Fab' fragments, one from a humanized anti-carcinoembryonic antigen antibody (hMN-14), and the other a murine antibody (679) against histamine-succinyl-glycine. These Fab' fragments were chemical conjugated to form a F(ab')2 that is joined by a stable thioether bond. The peptide used for these studies (IMP-241) contained two histamine-succinyl-glycine moieties for binding to the 679 portion of the bsMAb and a single 1,4,7,10-tetra-azacyclododecane N,N',N'',N'''-tetraacetic acid chelate for radiolabeling with 111In. Results: The bsMAb cleared rapidly in nude mice bearing the GW-39 human colonic cancer xenograft. Administration of a radiolabeled peptide 1 day after the bsMAb, using a bsMAb/peptide mole injection ratio of 10:1, allowed for higher tumor accretion than if delayed by 2 days. Tumor uptake measured 3 h after the peptide injection given 1 day after the bsMAb was 11.3±2.2% percentage of injected dose/g (%ID/g), with just 2.9±0.4% ID/g of the bsMAb in the tumor at this time. Tumor/blood ratios were 8.1±2.1. Peptide uptake was highest in the kidneys, but even so, the tumor/kidney ratio was 2.5±1.9 just 3 h after the peptide injection. Although low bsMAb/peptide mole injection ratios allow for greater concns. of the peptide in the tumor, kidney uptake is increased at a proportionally higher amount than in the tumor. Therefore, a bsMAb/peptide injection ratio of 10:1 with a 24-h interval was preferred for pretargeting. Increasing the bsMAb dose, and thereby increasing the bsMAb/peptide injection ratio, further enhanced the delivery of the radiolabeled peptide to the tumor, but the interval spacing between the bsMAb and peptide had to be increased. Despite having a lower %ID/g of the bsMAb in the tumor, with a bsMAb/peptide injection ratio of 50:1 and a 48-h interval, tumor uptake of the 111In-peptide was nearly 30% ID/g, a 1.6-fold improvement over that seen with the 10:1/24-h interval pretargeting group, and tumor/blood was 35:1, and tumor/kidney ratio was 8:1. Two fractionation strategies were also examined Giving two equal fractions of peptide after a single injection of bsMAb loaded more moles of peptide into the tumor but would not permit higher radioactivity delivery than what could be achieved with a single injection. However, area under the curve anal. indicated that giving repeated cycles of the bsMAb followed by the peptide would enable improvements in the amount of radioactivity delivered to the tumor without increasing the amount delivered to normal tissues, but the timing of the bsMAb/peptide cycles was important to optimize this process. Finally, it was noted that larger tumors (e.g. those > 0.3 g) were more likely to have higher peptide uptake in a pretargeting procedure than smaller tumors (e.g., those of .apprx.0.1 g), perhaps due to the greater mass of the bsMAb localized in the larger tumors, but also possibly because of better blood supply in these tumors. Conclusions: These studies reveal principles that might be applied generally to other pretargeting procedures and demonstrate how a bsMAb pretargeting method could potentially exceed a directly radiolabeled antibody in its ability to deliver radionuclides for cancer therapy.

L25 ANSWER 15 OF 62 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
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2003388504 EMBASE Development of new multivalent-bispecific agents for pretargeting tumor localization and therapy. Rossi E.A.; Sharkey R.M.; McBride W.; Karacay H.; Zeng L.; Hansen H.J.; **Goldenberg D.M.**; Chang C.-H.. C.-H. Chang, IBC Pharmaceuticals, Inc., 300 American Road, Morris Plains, NJ 07950, United States. kchang@immunomedics.com. Clinical Cancer Research 9/10 II (3886s-3896s) 1 Oct 2003.
Refs: 46.
ISSN: 1078-0432. CODEN: CCREF4. Pub. Country: United States. Language: English. Summary Language: English.

AB Purpose: Two bispecific diabodies (BS1.5 and BS1.5H) and two bispecific trivalent proteins (BS6 and BS8) were produced and tested as potential agents for pretargeted delivery of radiolabeled bivalent haptens to tumors expressing carcinoembryonic antigen. Experimental Design: Each of the four proteins was expressed in Escherichia coli and purified from the soluble fraction. BS1.5 and BS1.5H (a humanized version of BS1.5) were evaluated in the GW-39 human colonic tumor-nude mouse model using a di-HSG-1,4,7,10-tetra-azacyclododecane-N,N' ,N" N"'-tetraacetic acid peptide (IMP-241) radio-labeled with (111)In. The biodistribution and T/NT ratios were compared with those of hMN-14 x m679 (Fab' x Fab') prepared chemically. Results: In animals, both BS1.5 and BS1.5H cleared more rapidly than hMN-14 x m679 and showed tumor to nontumor ratios far superior to those of hMN-14 x m679. For example, with BS1.5 injected 8 h before (111)In-IMP-241, the tumor uptake of (111)In was 10.3 ± 2.7 and 6.3 ± 2.2% ID/g at 3 and 24 h, respectively, with the tumor to blood ratios being 167 ± 35 at 3 h and 631 ± 231 at 24 h. In comparison, the tumor to blood ratios of (111)In observed for hMN-14 ± m679 given 24 h earlier were 8 ± 2 at 3 h and 16 ± 3 at 24 h. Conclusions: These results indicate that BS1.5 and BS1.5H are promising candidates for use in a variety of pretargeting applications, including tumor therapy with radionuclides and drugs. BS6 and BS8 may be even more attractive because of their potential to achieve higher levels of tumor uptake because of divalent carcinoembryonic antigen binding.

L25 ANSWER 16 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
2003:847729 Document No. 140:252023 Development of new multivalent-bispecific agents for pretargeting tumor localization and therapy. Rossi, Edmund A.; Sharkey, Robert M.; McBride, William; Karacay, Habibe; Zeng, Li; Hansen, Hans J.; **Goldenberg, David M.**; Chang, Chien-Hsing (IBC Pharmaceuticals, Inc., Morris Plains, NJ, 07950, USA). Clinical Cancer Research, 9(10, Pt. 2), 3886s-3896s (English) 2003. CODEN: CCREF4. ISSN: 1078-0432. Publisher: American Association for Cancer Research.

AB Two bi-specific diabodies (BS1.5 and BS1.5H) and two bi-specific trivalent proteins (BS6 and BS8) were produced and tested as potential agents for pretargeted delivery of radiolabeled bivalent haptens to tumors expressing carcinoembryonic antigen. Each of the four proteins was expressed in Escherichia coli and purified from the soluble fraction. BS1.5 and BS1.5H (a humanized version of BS1.5) were evaluated in the GW-39 human colonic tumor-nude mouse model using a di-HSG-1,4,7,10-tetra-azacyclododecane-N,N',N'' N'''-tetraacetic acid peptide (IMP-241) radiolabeled with 111In. The biodistribution and T/NT ratios were compared with those of hMN-14 + m679 (Fab' + Fab') prepared chemical In animals, both BS1.5 and BS1.5H cleared more rapidly than hMN-14 + m679 and showed tumor to non-tumor ratios far superior to those of hMN-14 + m679. For example, with BS1.5 injected 8 h before 111In-IMP-241, the tumor uptake of 111In was 10.3 ± 2.7 and 6.3 ± 2.2% ID/g at 3 and 24 h, resp., with the tumor to blood ratios being 167 ± 35 at 3 h and 631 ± 231 at 24 h. In comparison, the tumor to blood ratios of 111In observed for hMN-14 + m679 given 24 h earlier were 8 ± 2 at 3 h and 16 ± 3 at 24 h. These results indicate that BS1.5 and BS1.5H are promising candidates for use in a variety of pretargeting applications, including tumor therapy with radionuclides and drugs. BS6 and BS8 may be even more attractive because of their potential to achieve higher levels of tumor uptake because of divalent carcinoembryonic antigen binding.

L25 ANSWER 17 OF 62 MEDLINE on STN DUPLICATE 5
2003445630. PubMed ID: 14506186. Pretargeting with labeled bivalent peptides allowing the use of four radionuclides: (111)In, (131)I, (99m)Tc, and (188)Re. van Schaijk Frank G; Oosterwijk Egbert; Soede Annemiele C; Oyen Wim J G; McBride William J; Griffiths Gary L; **Goldenberg David M**; Corstens Frans H M; Boerman Otto C. (University Medical Center Nijmegen, Nijmegen, the Netherlands.) Clinical cancer research : an official journal of the American Association for Cancer Research, (2003 Sep 1) 9 (10 Pt 2) 3880S-5S. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB PURPOSE: The therapeutic effect of directly labeled antibodies in solid tumors is limited, mainly due to the relatively low uptake of the radiolabeled antibody in tumors as compared with their blood level. In previous studies, we have shown that renal cell carcinoma (RCC) can be targeted very effectively with the (111)In-labeled bivalent peptide di-diethylenetriamminepentaacetic acid diDTPA-FKYK, after pretargeting the tumor with a **bispecific antibody.** In this study, we further developed this pretargeting approach for radioimmunotherapy of renal cell cancer. EXPERIMENTAL DESIGN: Pretargeting with the biologically produced anti-RCC x anti-DTPA bispecific monoclonal antibody (bsMAb G250xDTIn1) was tested in mice with SK-RC-52 RCC tumors. Tumors were pretargeted with 15 micro g of bispecific monoclonal antibody G250xDTIn1, and 24 h later, mice received 6 ng of the radiolabeled bivalent peptide. Two different peptides were used: (a) diDTPA-FKYK labeled with (111)In or (131)I; and (b) thiosemicarbonylglyoxylcysteinyl-diDTPA(In)-KYKK labeled with (99m)Tc or (188)Re. Mice were killed 6, 24, 48, and 72 h postinjection (p.i.), and biodistribution of the radiolabel was determined. RESULTS: The (111)In-labeled peptide showed excellent tumor uptake [42.6 +/- 7.3% injected dose/gram (ID/g) at 6 h p.i. and 25.6 +/- 7.7% ID/g at 72 h p.i.] and tumor:blood ratios (700 at 72 h p.i.). The specific tumor targeting of (188)Re- and (99m)Tc-labeled peptides was similar (20-25% ID/g, 6 h p.i.). However, the uptake and the retention in the tumor of the (99m)Tc- and (188)Re-labeled peptide were significantly lower than those of the (111)In-labeled peptide. Tumor uptake of the (131)I-labeled peptide was significantly lower as compared with the other three radiolabeled peptides; furthermore, an almost complete washout of the radiolabel from the tumor over time was observed (14.5 +/- 4.9% ID/g at 6 h p.i. and 0.33 +/- 0.15% ID/g at 72 h p.i.). CONCLUSIONS: Using a newly developed bivalent peptide, this pretargeting approach can now be used for targeting with the matched pair (188)Re and (99m)Tc.

L25 ANSWER 18 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN

2003:847728 Document No. 141:49666 Pretargeting with labeled bivalent peptides allowing the use of four radionuclides: 111In, 131I, 99mTc, and 188Re. van Schaijk, Frank G.; Oosterwijk, Egbert; Soede, Annemiele C.; Oyen, Wim J. G.; McBride, William J.; Griffiths, Gary L.; **Goldenberg, David M.**; Corstens, Frans H. M.; Boerman, Otto C. (University Medical Center Nijmegen, Nijmegen, Neth.). Clinical Cancer Research, 9(10, Pt. 2), 3880s-3885s (English) 2003. CODEN: CCREF4. ISSN: 1078-0432. Publisher: American Association for Cancer Research.

AB Purpose: The therapeutic effect of directly labeled antibodies in solid tumors is limited, mainly due to the relatively low uptake of the radiolabeled antibody in tumors as compared with their blood level. In previous studies, we have shown that renal cell carcinoma (RCC) can be targeted very effectively with the 111In-labeled bivalent peptide didiethylenetriamminepentaacetic acid diDTPA-FKYK, after pretargeting the tumor with a **bispecific antibody.** In this study, we further developed this pretargeting approach for radioimmunotherapy of renal cell cancer. Exptl. Design: Pretargeting with the biol. produced anti-RCC + anti-DTPA bispecific monoclonal antibody (bsMAb G250xDTIn1) was tested in mice with SKRC-52 RCC tumors. Tumors were pretargeted with 15 μg of bispecific monoclonal antibody G250xDTIn1, and 24 h later, mice received 6 ng of the radiolabeled bivalent peptide. Two different peptides were used: (a) diDTPA-FKYK labeled with 111In or 131I; and (b) thiosemicarbonylglyoxylcysteinyl-diDTPA(In)-KYKK labeled with 99mTc or 188Re. Mice were killed 6, 24, 48, and 72 h postinjection (p.i.), and biodistribution of the radiolabel was determined Results: The 111In-labeled peptide showed excellent tumor uptake [42.6±7.3% injected dose/g (ID/g) at 6 h p.i. and 25.6±7.7% ID/g at 72 h p.i.] and tumor:blood ratios (700 at 72 h p.i.). The specific tumor targeting of 188Re- and 99mTc-labeled peptides was similar (20-25% ID/g, 6 h p.i.). However, the uptake and the retention in the tumor of the 99mTc- and 188Re-labeled peptide were significantly lower than those of the 111In-labeled peptide. Tumor uptake of the 131I-labeled peptide was significantly lower as compared with the other three radiolabeled peptides; furthermore, an almost complete washout of the radiolabel from the tumor over time was observed (14.5±4.9% ID/g at 6 h p.i. and 0.33±0.15% ID/g at 72 h p.i.). Conclusions: Using a newly developed bivalent peptide, this pretargeting approach can now be used for targeting with the matched pair 188Re and 99mTc.

L25 ANSWER 19 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on

STN
2003:503501 Document No.: PREV200300498922. A pretargeted, **bispecific**
antibody sytem for nuclear imaging of pancreatic cancer. Gold,
David V. [Reprint Author]; Cardillo, Thomas M.; Karacay, Habibe; Sharkey,
Robert M.; Chang, Ken; **Goldenberg, David M.**. Garden State Cancer
Center, Belleville, NJ, USA. Proceedings of the American Association for
Cancer Research Annual Meeting, (July 2003) Vol. 44, pp. 1273. print.
Meeting Info.: 94th Annual Meeting of the American Association for Cancer
Research. Washington, DC, USA. July 11-14, 2003.
ISSN: 0197-016X. Language: English.

L25 ANSWER 20 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on
STN
2003:502384 Document No.: PREV200300498381. Generation and characteristics of
a mouse monoclonal anti-DOTA antibody, LG-1, for a **bispecific**
antibody (bsMAb) pretargeting system. Losman, Michele Jeanne
[Reprint Author]; Govindan, S. V.; Newsome, G. A.; Karacay, H.; McBride,
W. J.; Chang, K.; Griffiths, G. L.; Hansen, H. J.; **Goldenberg, D.**
M.; Sharkey, R. M.. Immunomedics, Morris Plains, NJ, USA. Proceedings
of the American Association for Cancer Research Annual Meeting, (July
2003) Vol. 44, pp. 1121. print.
Meeting Info.: 94th Annual Meeting of the American Association for Cancer
Research. Washington, DC, USA. July 11-14, 2003.
ISSN: 0197-016X. Language: English.

L25 ANSWER 21 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation.
on STN
2003:504407 The Genuine Article (R) Number: 688EY. Radioimmunotherapy: is
avidin-biotin pretargeting the preferred choice among pretargeting
methods? Against. **Goldenberg D M (Reprint)**; Sharkey R M;
Karacay H; **Goldenberg D M**; McBride W; Hansen H J;
Goldenberg D M; Chang C H; Rossi E A; Chatal J F; Barbet J. Garden
State Canc Ctr, Ctr Mol Med & Immunol, 520 Belleville Ave, Belleville, NJ
07109 USA (Reprint); Garden State Canc Ctr, Ctr Mol Med & Immunol,
Belleville, NJ 07109 USA; Immunomed Inc, Morris Plains, NJ USA; IBC
Pharmaceut Inc, Morris Plains, NJ USA; Ctr Rene Gauducheau, F-44035
Nantes, France; INSERM, U463, Nantes, France. EUROPEAN JOURNAL OF NUCLEAR
MEDICINE AND MOLECULAR IMAGING (MAY 2003) Vol. 30, No. 5, pp. 776-780.
Publisher: SPRINGER-VERLAG. 175 FIFTH AVE, NEW YORK, NY 10010 USA. ISSN:
1619-7070. Pub. country: USA; France. Language: English.

L25 ANSWER 22 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on
STN DUPLICATE 6
2003:402879 Document No.: PREV200300402879. Optimizing **bispecific**
antibody (bsMAb) pretargeting for cancer radioimmunotherapy.
Sharkey, R. M. [Reprint Author]; Karacay, H.; McBride, W.; Chang, C. H.;
Hansen, H. J.; **Goldenberg, D. M.**. Molecular Imaging, Center for
Molecular Medicine and Immunology, Belleville, NJ, USA. Journal of Nuclear
Medicine, (May 2003) Vol. 44, No. 5 Supplement, pp. 362P. print.
Meeting Info.: 50th Annual Meeting of the Society of Nuclear Medicine. New
Orleans, LA, USA. June 21-25, 2003. Society of Nuclear Medicine.
ISSN: 0161-5505 (ISSN print). Language: English.

L25 ANSWER 23 OF 62 MEDLINE on STN DUPLICATE 7
2003035973. PubMed ID: 12543788. A universal pretargeting system for
cancer detection and therapy using **bispecific antibody**
. Sharkey Robert M; McBride William J; Karacay Habibe; Chang Ken;
Griffiths Gary L; Hansen Hans J; **Goldenberg David M**. (Center for
Molecular Medicine and Immunology, Belleville, New Jersey 07109, USA.)
Cancer research, (2003 Jan 15) 63 (2) 354-63. Journal code: 2984705R.
ISSN: 0008-5472. Pub. country: United States. Language: English.
AB Multistep targeting systems represent highly selective alternatives to
targeting systems using directly radiolabeled antibodies for diagnostic
and therapeutic applications. A flexible **bispecific**
antibody (bsMAb) multistep, pretargeting system that potentially

can be developed for use with a variety of different imaging or therapeutic agents is described herein. The flexibility of this system is based on use of an antibody directed against histamine-succinyl-glycine (HSG) and the development of peptides containing the HSG residue. HSG-containing peptides were synthesized with either 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid for the chelation of 111In, 90Y, or 177Lu, or a technetium/rhenium chelate. The peptides can be radiolabeled to a high specific activity in a facile manner that avoids the need for purification. In vivo studies in nude mice bearing human colon tumor xenografts showed that the radiolabeled peptides cleared rapidly from the body with minimal retention in tumor or normal tissues. For pretargeting, these peptides were used in combination with a bsMAb composed of the anti-HSG Fab' that was covalently coupled with the Fab' of either an anticarcinoembryonic antigen or an anticolon-specific antigen-p antibody to provide tumor targeting capability. When the radiolabeled peptides were administered 1-2 days after a pretargeting dose of the bsMAbs, tumor uptake of the radiolabeled peptides increased as much as 28-175-fold over that seen with the peptides alone with tumor:nontumor ratios exceeding 2:1 to 8:1 within just 3 h of the peptide injection, which was a marked improvement over the tumor:nontumor ratios seen with a directly radiolabeled 99mTc-anti-anticarcinoembryonic antigen Fab' at this same time. The anticolon-specific antigen-p x anti-HSG F(ab')2 bsMAb had the highest and longest retention in the tumor, and when used in combination with the 111In-labeled peptide, radiation dose estimates for therapeutic radionuclides, such as 90Y and 177Lu, suggested that antitumor effects would be expected with tolerable radiation exposure to the normal tissues. These results suggest that this multistep, pretargeting system has diagnostic imaging and therapeutic potential.

L25 ANSWER 24 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 8
2003:383427 Document No.: PREV200300383427. Development of gallium-complexed bivalent hapten-peptide conjugates for use with **bispecific antibody** pretargeting in positron emission tomography (PET). Griffiths, G. L. [Reprint Author]; McBride, W. J.; Sheerin, A. A.; Sharkey, R. M.; Karacay, H.; Hansen, H. J.; Horak, I. D.; **Goldenberg, D. M.**. Research and Development, Immunomedics, Inc., Morris Plains, NJ, USA. Journal of Nuclear Medicine, (May 2003) Vol. 44, No. 5 Supplement, pp. 295P. print.
Meeting Info.: 50th Annual Meeting of the Society of Nuclear Medicine. New Orleans, LA, USA. June 21-25, 2003. Society of Nuclear Medicine.
ISSN: 0161-5505 (ISSN print). Language: English.

L25 ANSWER 25 OF 62 MEDLINE on STN DUPLICATE 9
2003181188. PubMed ID: 12700944. Advancing role of radiolabeled antibodies in the therapy of cancer. **Goldenberg David M.** (Garden State Cancer Center, Center for Molecular Medicine and Immunology, Belleville, NJ 07109, USA.. dmg.gscancer@att.net) . Cancer immunology, immunotherapy : CII, (2003 May) 52 (5) 281-96. Ref: 139. Journal code: 8605732. ISSN: 0340-7004. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB This review focuses on the use of radiolabeled antibodies in the therapy of cancer, termed radioimmunotherapy (RAIT). Basic problems concerned with the choice of antibody, radionuclide, and physiology of the tumor and host are discussed, followed by a review of the pertinent clinical publications of various radioantibody constructs in the treatment of hematopoietic and solid tumors of diverse histopathology, grade, and stage, and in different clinical settings. Factors such as dose rate delivered, tumor size, and radiosensitivity play a major role in determining therapeutic response, while target-to-nontarget ratios and, particularly, circulating radioactivity to the bone marrow determine the principal dose-limiting toxicities. RAIT appears to be gaining a place in the therapy of hematopoietic neoplasms, such as non-Hodgkin's lymphoma: several agents are advancing in clinical trials toward registration, and one has recently been approved by the FDA. Although RAIT of solid tumors has shown less progress, use of pretargeting strategies, such as an affinity-enhancement system consisting of **bispecific antibodies** separating targeting from delivery of the radiotherapeutic, appears to enhance tumor-to-nontumor ratios, and may increase radiation doses to tumors more selectively than directly labeled antibodies.

L25 ANSWER 26 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 10

2003:360460 Document No.: PREV200300360460. Pretargeting with **bispecific antibody** as a method for improved imaging of pancreatic cancer. Karacay, H. [Reprint Author]; Cardillo, T.; McBride, W. J.; Gold, D. V.; Sharkey, R. M.; Chang, C. H.; Hansen, H. J.; **Goldenberg, D. M.**. Chemistry, Garden State Cancer Center, Belleville, NJ, USA. Journal of Nuclear Medicine, (May 2003) Vol. 44, No. 5 Supplement, pp. 176P. print.
Meeting Info.: 50th Annual Meeting of the Society of Nuclear Medicine. New Orleans, LA, USA. June 21-25, 2003. Society of Nuclear Medicine.
ISSN: 0161-5505 (ISSN print). Language: English.

L25 ANSWER 27 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:377770 Document No.: PREV200200377770. Method of treatment with a non-antigenic toxin-conjugate and fusion protein of internalizing receptor system. **Goldenberg, David M.** [Inventor]. ASSIGNEE: Immunomedics, Inc.. Patent Info.: US 6399068 June 04, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (June 4, 2002) Vol. 1259, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB A conjugate of a toxin and a cytokine, and a fusion protein comprising a **bispecific antibody** that has a first specificity for a cell marker specific to a malignant cell and a second specificity for a region of IL-15alpha, each optionally further comprising a radionuclide, are useful therapeutic reagents for treating leukemias and lymphomas.

L25 ANSWER 28 OF 62 MEDLINE on STN DUPLICATE 11

2002475152. PubMed ID: 12236788. Pretargeting for cancer radioimmunotherapy with **bispecific antibodies**: role of the **bispecific antibody**'s valency for the tumor target antigen. Karacay H; Sharkey R M; McBride W J; Griffiths G L; Qu Z; Chang K; Hansen H J; **Goldenberg D M.** (Center for Molecular Medicine and Immunology, Belleville, New Jersey 07109, USA.) Bioconjugate chemistry, (2002 Sep-Oct) 13 (5) 1054-70. Journal code: 9010319. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB The use of a divalent effector molecule improves **bispecific antibody** (bsMAb) pretargeting by enabling the cross-linking of monovalently bound bsMAb on the cell surface, thereby increasing the functional affinity of a bsMAb. In this work, it was determined if a bsMAb with divalency for the primary target antigen would improve bsMAb pretargeting of a divalent hapten. The pretargeting of a (99m)Tc-labeled divalent DTPA-peptide, IMP-192, using a bsMAb prepared by chemically coupling two Fab' fragments, one with monovalent specificity to the primary target antigen, carcinoembryonic antigen (CEA), and to indium-loaded DTPA [DTPA(In)], was compared to two other bsMAbs, both with divalency to CEA. One conjugate used the whole anti-CEA IgG, while the other used the anti-CEA F(ab')(2) fragment to make bsMAbs that had divalency to CEA, but with different molecular weights to affect their pharmacokinetic behavior. The rate of bsMAb blood clearance was a function of molecular weight (IgG x Fab' < F(ab')(2) x Fab' < Fab' x Fab' conjugate). The IgG x Fab' bsMAb conjugate had the highest uptake and longest retention in the tumor. However, when used for pretargeting, the F(ab')(2) x Fab' conjugate allowed for superior tumor accretion of the (99m)Tc-IMP-192 peptide, because its more rapid clearance from the blood enabled early intervention with the radiolabeled peptide when tumor uptake of the bsMAb was at its peak. Excellent peptide targeting was also seen with the Fab' x Fab' conjugate, albeit tumor uptake was lower than with the F(ab')(2) x Fab' conjugate. Because the IgG x Fab' bsMAb cleared from the blood so slowly, when the peptide was given at the time of its maximum tumor accretion, the peptide was captured predominantly by the bsMAb in the blood. Several strategies were explored to reduce the IgG x Fab' bsMAb remaining in the blood to take advantage of its 3-4-fold higher tumor accretion than the other bsMAb conjugates. A number of agents were tested, including those that could clear the bsMAb from the blood (e.g., galactosylated or nongalactosylated anti-id antibody) and those that could block the anti-DTPA(In) binding arm [e.g., DTPA(In), divalent-DTPA(In) peptide, and DTPA coupled to bovine serum albumin (BSA) or IgG]. When clearing agents were given 65 h after the IgG x Fab' conjugate (time of maximum tumor accretion for this bsMAb), (99m)Tc-IMP-192 levels in the blood were significantly reduced, but a majority of the peptide localized in the liver. Increasing the interval between the clearing agent and the time the peptide was given to allow for further processing of the bsMAb-clearing agent complex did not improve targeting. At the dose and level of substitution tested, galacosylated BSA-DTPA(In) was cleared too quickly to be an effective blocking agent, but BSA- and IgG-DTPA(In) conjugates were able to reduce the uptake of the (99m)Tc-IMP-192 in the blood and liver. Tumor/nontumor ratios compared favorably for the radiolabeled peptide using the IgG x Fab'/blocking agent combination and the F(ab')(2) x Fab' (no clearing/blocking agent), and peptide uptake 3 h after the blocking agent even exceeded that of the F(ab')(2) x Fab'. However, this higher level of peptide in the tumor was not sustained over 24 h, and actually decreased to levels lower than that seen with the F(ab')(2) x Fab' by this time. These results demonstrate that divalency of a bsMAb to its primary target antigen can lead to higher tumor accretion by a pretargeted divalent peptide, but that the pharmacokinetic behavior of the bsMAb also needs to be optimized to allow for its clearance from the blood. Otherwise, blocking agents will need to be developed to reduce unwanted peptide uptake in normal tissues.

L25 ANSWER 29 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2002:418996 Document No.: PREV200200418996. Comparison of **bispecific antibodies** for pretargeted delivery of small molecules to tumors. Hayes, Marianne K. [Reprint author]; Ma, Hong [Reprint author]; Consolino, Kim J. [Reprint author]; Tomko, Richard P. [Reprint author]; Wang, Ching Y. [Reprint author]; Hansen, Hans J. [Reprint author]; **Goldenberg, David M.** [Reprint author]; Qu, Zhengxing [Reprint author]. Immunomedics Inc, Morris Plains, NJ, USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2002) Vol. 43, pp. 969. print.
Meeting Info.: 93rd Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA. April 06-10, 2002.
ISSN: 0197-016X. Language: English.

L25 ANSWER 30 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2002:409872 Document No.: PREV200200409872. A new **bispecific antibody** pretargeting system suitable for therapy with 90Y- or 177Lu-labeled peptides. Sharkey, Robert M. [Reprint author]; Karacay, H.; McBride, W.; Hansen, H. J.; Chang, K.; Zeng, L.; Losman, M.; Richel, H.; **Goldenberg, D. M.**. Garden State Cancer Center, Belleville, NJ, USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2002) Vol. 43, pp. 913. print.
Meeting Info.: 93rd Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA. April 06-10, 2002.
ISSN: 0197-016X. Language: English.

L25 ANSWER 31 OF 62 MEDLINE on STN DUPLICATE 12
2002255245. PubMed ID: 11994535. Targeted therapy of cancer with radiolabeled antibodies. **Goldenberg David M.** (Garden State Cancer Center, Center for Molecular Medicine and Immunology, Belleville,

New Jersey 07109, USA.. dmg.gscancer@worldnet.att.net) . Journal of nuclear medicine : official publication, Society of Nuclear Medicine, (2002 May) 43 (5) 693-713. Ref: 205. Journal code: 0217410. ISSN: 0161-5505. Pub. country: United States. Language: English.

AB This review focuses on the use of radiolabeled antibodies in the therapy of cancer, termed radioimmunotherapy (RAIT). Basic problems concerning the choice of antibody and radionuclide and the physiology of tumor and host are discussed. Then follows a review of pertinent clinical publications on various radioantibody constructs in the treatment of hematopoietic and solid tumors of diverse histopathologies, grades, and stages, and in different clinical settings. Factors such as dose rate delivered, tumor size, and radiosensitivity play a major role in determining therapeutic response, while target-to-nontarget ratios and, particularly, circulating radioactivity to the bone marrow determine the major dose-limiting toxicities. RAIT appears to be gaining a place in the therapy of hematopoietic neoplasms, such as non-Hodgkin's lymphoma, with several agents advancing in clinical trials toward registration, of which one has just been approved by the FDA. Although RAIT of solid tumors has shown less progress, pretargeting strategies, such as an affinity-enhancement system consisting of **bispecific antibodies** separating targeting from delivery of the radiotherapeutic, appear to enhance tumor-to-nontumor ratios and may increase rad doses to tumor more selectively than directly labeled antibodies.

L25 ANSWER 32 OF 62 MEDLINE on STN DUPLICATE 13

2002716941. PubMed ID: 12479274. Molecular advances in pretargeting radioimunotherapy with **bispecific antibodies.** Chang Chien-Hsing; Sharkey Robert M; Rossi Edmund A; Karacay Habibe; McBride William; Hansen Hans J; Chatal Jean-Francois; Barbet Jacques; **Goldenberg David M.** (IBC Pharmaceuticals, L. L. C., Morris Plains, New Jersey 07950, USA.) Molecular cancer therapeutics, (2002 May) 1 (7) 553-63. Ref: 72. Journal code: 101132535. ISSN: 1535-7163. Pub. country: United States. Language: English.

AB The use of antibodies against tumor-associated cell surface antigens for the targeted delivery of radionuclides was introduced >20 years ago. Although encouraging results have been achieved with radiolabeled antibodies in the management of hematopoietic malignancies, there remains a need for successfully treating solid tumors with this modality. One promising approach involving pretargeted delivery of radionuclides has been shown to be capable of significantly increasing the radioactive uptake in tumor relative to normal organs, thereby potentially improving the efficacy of both detection and therapy of cancer. Uncoupling of the radionuclide from the tumor-targeting antibody allows the relatively slow process of antibody localization and clearance to occur before a very rapid and highly specific delivery of the radioactive payload carried on a small molecule, such as a peptide. This minireview discusses the various strategies and advancements made since the concept of pretargeting was proposed in the mid-1980s, with emphasis on those comprising **bispecific antibodies** for cancer therapy. Critical aspects of these pretargeting systems for achieving higher tumor:nontumor ratios are considered. In addition, both preclinical and clinical results obtained from a pretargeting method known as the Affinity Enhancement System are presented. Future directions of pretargeting technology are also suggested.

L25 ANSWER 33 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 14

2002:474344 Document No.: PREV200200474344. A pretargeting, **bispecific antibody** method for improved imaging and therapy of pancreatic cancer. Karacy, H. [Reprint author]; Cardillo, T.; McBride, W. J.; Gold, D. V.; Sharkey, R. M.; Chang, K.; Hansen, H. J.; **Goldenberg, D. M.** . Garden State Cancer Center, Belleville, NJ, USA. Journal of Nuclear Medicine, (May, 2002) Vol. 43, No. 5 Supplement, pp. 369P. print. Meeting Info.: 49th Annual Meeting of the Society of Nuclear Medicine. Los

Angeles, CA, USA. June 15-19, 2002.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 34 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 15
2002:456088 Document No.: PREV200200456088. Pharmacokinetics and biodistribution of anti-CEAXanti-hapten **bispecific antibody** (BsMAb) and 131I-labeled hapten in pretargeted radioimmunotherapy patients. Barbet, J. [Reprint author]; Kraeber-Bodere, F.; Faivre-Chauvet, A.; Ferrer, L.; Rousseau, C.; Resche, I.; Vuillez, J. P.; Devillers, A.; Chang, K.; Sharkey, R. M.; **Goldenberg, D. M.**; Chatal, J. F.. Inserm U463, Nantes, France. Journal of Nuclear Medicine, (May, 2002) Vol. 43, No. 5 Supplement, pp. 159P. print.
Meeting Info.: 49th Annual Meeting of the Society of Nuclear Medicine. Los Angeles, CA, USA. June 15-19, 2002.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 35 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2002:456068 Document No.: PREV200200456068. Therapeutic results using a novel **bispecific antibody** pretargeting system for the delivery of 90Y. Sharkey, R. M. [Reprint author]; Karacay, H.; McBride, W. J.; Chang, K.; Hansen, H. J.; **Goldenberg, D. M.**. Garden State Cancer Center, Belleville, NJ, USA. Journal of Nuclear Medicine, (May, 2002) Vol. 43, No. 5 Supplement, pp. 154P. print.
Meeting Info.: 49th Annual Meeting of the Society of Nuclear Medicine. Los Angeles, CA, USA. June 15-19, 2002.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 36 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2002:442231 The Genuine Article (R) Number: 551KL. Therapeutic results using a novel **bispecific antibody** pretargeting system for the delivery of Y-90.. Sharkey Y R M (Reprint); Karacay H; McBride W J; Chang K; Hansen H J; **Goldenberg D M.** Garden State Canc Ctr, Belleville, NJ USA; Immunomed Inc, Morris Plains, NJ USA; LLC, IBC Pharmaceut, Morris Plains, NJ USA. JOURNAL OF NUCLEAR MEDICINE (MAY 2002) Vol. 43, No. 5, Supp. [S], pp. 154P-154P. MA 559. Publisher: SOC NUCLEAR MEDICINE INC. 1850 SAMUEL MORSE DR, RESTON, VA 20190-5316 USA. ISSN: 0161-5505. Pub. country: USA. Language: English.

L25 ANSWER 37 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 16
2002:456067 Document No.: PREV200200456067. Tumor targeting with bivalent peptides labeled with four different radionuclides following pretargeting with a **bispecific antibody.** Van Schaijk, F. G. [Reprint author]; Soede, A. C.; McBride, W. J.; Griffiths, G. L.; Oosterwijk, E.; **Goldenberg, D. M.**; Corstens, F. H.; Boerman, O. C.. University Medical Center Nijmegen, Nijmegen, Netherlands. Journal of Nuclear Medicine, (May, 2002) Vol. 43, No. 5 Supplement, pp. 153P-154P. print.
Meeting Info.: 49th Annual Meeting of the Society of Nuclear Medicine. Los Angeles, CA, USA. June 15-19, 2002.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 38 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2001:940018 The Genuine Article (R) Number: 491RE. Clinical studies of pretargeted, two-step radioimmunotherapy (RAIT) with **bispecific antibody** (BsMAb) and 131I-labeled bivalent hapten in patients with CEA-expressing cancers.. Chatal J (Reprint); Kraeber-Bodere F; Faivre-Chauvet A; Cerato E; Devellers A; Sharkey R M; Chang K; Barbet J; **Goldenberg D M.** INSERM, Nantes, France; Garden State Canc Ctr, Belleville, NJ USA; IBC Pharmaceut, LLC, Morris Plains, NJ USA. CLINICAL CANCER RESEARCH (NOV 2001) Vol. 7, No. 11, Supp. [S], pp. 3787S-3787S. MA

663. Publisher: AMER ASSOC CANCER RESEARCH. PO BOX 11806, BIRMINGHAM, AL 35202 USA. ISSN: 1078-0432. Pub. country: France; USA. Language: English.

L25 ANSWER 39 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:940015 The Genuine Article (R) Number: 491RE. A novel, recombinant, **bispecific antibody** pretargeting system for cancer radioimmunotherapy.. Sharkey R M (Reprint); Rossi E; Karacay H; McBride W; Chang K; Zang L; Qu T; Griffiths G; Hansen H J; **Goldenberg D M***. Garden State Canc Ctr, Belleville, NJ USA; IBC Pharmaceut, LLC, Morris Plains, NJ USA; Immunomed Inc, Morris Plains, NJ USA. CLINICAL CANCER RESEARCH (NOV 2001) Vol. 7, No. 11, Supp. [S], pp. 3786S-3786S. MA 660. Publisher: AMER ASSOC CANCER RESEARCH. PO BOX 11806, BIRMINGHAM, AL 35202 USA. ISSN: 1078-0432. Pub. country: USA. Language: English.**

L25 ANSWER 40 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 17

2001:561399 Document No.: PREV200100561399. Development of a novel "universal" ***bispecific antibody pretargeting system for imaging and therapy. Sharkey, R. M. [Reprint author]; Karacay, H.; McBride, W.; Chang, K.; Zheng, L.; Losman, M.; Griffiths, G.; Barbet, J.; Hansen, H.; **Goldenberg, D. M.**. Garden State Cancer Center, Belleville, NJ, USA. Journal of Nuclear Medicine, (May, 2001) Vol. 42, No. 5 Supplement, pp. 148P. print.
Meeting Info.: 48th Annual Meeting of the Society of Nuclear Medicine. Toronto, Ontario, Canada. June 23-27, 2001.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 41 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 18

2001:555257 Document No.: PREV200100555257. Clinical optimization of two-step radioimmunotherapy (RAIT) using an 131I-labeled anti-CEA/anti-DTPA **bispecific antibody** and an 131I-labeled bivalent DTPA hapten in patients with CEA-expressing tumor. Kraeber-Bodere, F. [Reprint author]; Faivre-Chauvet, A.; Cerato, E.; Devillers, A.; Sharkey, R. M.; Chang, K.; **Goldenberg, D. M.**; Barbet, J.; Chatal, J. F.. Rene Gaudicheau Cancer Centre, Nantes, France. Journal of Nuclear Medicine, (May, 2001) Vol. 42, No. 5 Supplement, pp. 123P. print.
Meeting Info.: 48th Annual Meeting of the Society of Nuclear Medicine. Toronto, Ontario, Canada. June 23-27, 2001.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 42 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:369600 Document No.: PREV200100369600. Evaluation of clearing and blocking agents for improving tumor/non-tumor ratios in **bispecific antibody** pretargeting. Karacay, Habibe [Reprint author]; Sharkey, Robert M.; McBride, William; Chang, C.-H. Ken; Griffiths, Gary L.; Hansen, Hans J.; **Goldenberg, David M.**. Garden State Cancer Center, Belleville, NJ, USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2001) Vol. 42, pp. 94. print.
Meeting Info.: 92nd Annual Meeting of the American Association for Cancer Research. New Orleans, LA, USA. March 24-28, 2001. American Association for Cancer Research.
ISSN: 0197-016X. Language: English.

L25 ANSWER 43 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:173541 Document No.: PREV200100173541. Non-antigenic toxin-conjugate and fusion protein of internalizing receptor system. **Goldenberg, David M.** [Inventor, Reprint author]. Mendham, NJ, USA. ASSIGNEE: Immunomedics, Inc.. Patent Info.: US 6083477 July 04, 2000. Official Gazette of the United States Patent and Trademark Office Patents, (July 4, 2000) Vol. 1236, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB A conjugate of a toxin and a cytokine, and a fusion protein comprising a **bispecific antibody** that has a first specificity for a cell marker specific to a malignant cell and a second specificity for a region of IL-15alpha, each optionally further comprising a radionuclide, are useful therapeutic reagents for treating leukemias and lymphomas.

L25 ANSWER 44 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
2000:880995 Document No. 134:41104 Immunotherapy of autoimmune disorders using antibodies which target B-cells. **Goldenberg, David M.**; Hansen, Hans J. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000074718 A1 20001214, 39 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US15780 20000609. PRIORITY: US 1999-PV138284 19990609.

AB Antibodies that bind with a B-cell antigen provide an effective means to treat autoimmune disorders. Antibodies and fragments, which may be conjugated or naked, are used alone or in multimodal therapies. The antibodies may be **bispecific antibodies** which may be produced recombinantly as fusion proteins, or as hybrid, polyspecific antibodies.

L25 ANSWER 45 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. **Goldenberg, David M.** (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug transporter protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L25 ANSWER 46 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:900799 The Genuine Article (R) Number: 364KW. Pretargeting colonic tumors with engineered **bispecific antibodies** for improved radioimmunotherapy (RAIT).. Leung S O (Reprint); Qu Z; Sharkey R M; McBride W J; Losman M J; Chang C H; Barbet J; Karacay H; **Goldenberg D M**; Hansen H J. IBC PHARMACEUT LLC, MORRIS PLAINS, NJ; GARDEN STATE CANC CTR, BELLEVILLE, NJ; IMMUNOMED INC, MORRIS PLAINS, NJ. JOURNAL OF NUCLEAR MEDICINE (MAY 2000) Vol. 41, No. 5, Supp. [S], pp. 1185-1185. Publisher: SOC NUCLEAR MEDICINE INC. 1850 SAMUEL MORSE DR, RESTON, VA 20190-5316. ISSN: 0161-5505. Pub. country: USA. Language: English.

L25 ANSWER 47 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:900636 The Genuine Article (R) Number: 364KW. Site-specific delivery of small molecules to tumors via **bispecific antibody** tumor pretargeting: The use of imaging isotopes in developmental studies.. McBride W J (Reprint); **Goldenberg D M**; Karacay H; Hansen H J;

Griffiths G L. GARDEN STATE CANC CTR, BELLEVILLE, NJ; IMMUNOMED INC, MORRIS PLAINS, NJ. JOURNAL OF NUCLEAR MEDICINE (MAY 2000) Vol. 41, No. 5, Supp. [S], pp. 1023-1023. Publisher: SOC NUCLEAR MEDICINE INC. 1850 SAMUEL MORSE DR, RESTON, VA 20190-5316. ISSN: 0161-5505. Pub. country: USA. Language: English.

L25 ANSWER 48 OF 62 MEDLINE on STN DUPLICATE 19

2001084538. PubMed ID: 11087333. Experimental pretargeting studies of cancer with a humanized anti-CEA x murine anti-[In-DTPA] **bispecific antibody** construct and a (99m)Tc-/(188)Re-labeled peptide. Karacay H; McBride W J; Griffiths G L; Sharkey R M; Barbet J; Hansen H J; **Goldenberg D M.** (Immunomedics, Inc., Morris Plains, New Jersey, Garden State Cancer Center, Belleville, New Jersey, USA.) Bioconjugate chemistry, (2000 Nov-Dec) 11 (6) 842-54. Journal code: 9010319. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB The aim of this study was to localize (99m)Tc and (188)Re radionuclides to tumors, using a **bispecific antibody** (bsMAb) in a two-step approach where the radionuclides are attached to novel peptides incorporating moieties recognized by one arm of the bsMAb. A chemically cross-linked human/murine bsMAb, hMN-14 x 734 (Fab' x Fab'), anti-carcinoembryonic antigen [CEA] x anti-indium-DTPA was prepared as a prelude to constructing a fully humanized bsMAb for future clinical application. N,N'-o-Phenylenedimaleimide was used to cross-link the Fab' fragments of the two antibodies at their hinge regions. This construct was shown to be >92% pure and fully reactive with CEA and a divalent (indium)DTPA-peptide. For pretargeting purposes, a peptide, IMP-192 [Ac-Lys(In-DTPA)-Tyr-Lys(In-DTPA)-Lys(TscG-Cys-)-NH(2) TscG = 3-thiosemicarbazonylglyoxyl], with two indium-DTPAs and a chelate for selectively binding (99m)Tc or (188)Re, was synthesized. IMP-192 was formulated in a "single dose" kit and later radiolabeled with (99m)Tc (94-99%) at up to 1836 Ci/mmol and with (188)Re (97%) at 459-945 Ci/mmol of peptide. [(99m)Tc]IMP-192 was shown to be stable by extensive in vitro and in vivo testing and had no specific uptake in the tumor with minimal renal uptake. The biodistribution of the hMN-14 x murine 734 bsMAb was compared alone and in a pretargeting setting to a fully murine anti-CEA (F6) x 734 bsMAb that was reported previously [Gautherot, E., Bouhou, J., LeDoussal, J.-M., Manetti, C., Martin, M., Rouvier, E., and Barbet, J. (1997) Therapy for colon carcinoma xenografts with **bispecific antibody**-targeted, iodine-131-labeled bivalent hapten. Cancer 80 (Suppl.), 2618-2623]. Both bsMAbs maintained their integrity and dual binding specificity in vivo, but the hMN-14 x m734 was cleared more rapidly from the blood. This coincided with an increased uptake of the hMN-14 x m734 bsMAb in the liver and spleen, suggesting an active reticuloendothelial cell recognition mechanism of this mixed species construct in naive mice. Animals bearing GW-39 human colonic cancer xenografts were injected with bsMAb (15 microg) and after allowing 24 or 72 h for the bsMAb constructs to clear from the blood (hMN-14 and murine F6 x 734, respectively), [(188)Re]IMP-192 (7 microCi) or [(99m)Tc]IMP-192 (10 microCi) was injected at a bsMAb:peptide ratio of 10:1. Tumor uptake of [(99m)Tc] or [(188)Re]IMP-192 was 12.6 +/- 5.2 and 16.9 +/- 5.5% ID/g at 3 h postinjection, respectively. Tumor/nontumor ratios were between 5.6 and 23 to 1 for every major organ, indicating that early imaging with (99m)Tc will be possible. Radiation absorbed doses showed a 4.8-, 7.2-, and a 12.6 to 1.0 tumor to blood, kidney, and liver ratios when (188)Re was used. Although this new bsMAb pretargeting approach requires further optimization, it already shows very promising targeting results for both radioimmunodetection and radioimmunotherapy of colorectal cancer.

L25 ANSWER 49 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2000:264579 Document No.: PREV200000264579. Humanized anti-CEA X chimeric anti-(indium-DTPA) **bispecific antibody** conjugates for radioimmunotherapy of CEA-positive tumors. Karacay, Habibe [Reprint author]; McBride, W.; Sharkey, R. M.; Hansen, H.; **Goldenberg, D.**

M.. Golden State Cancer Ctr, Belleville, NJ, USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2000) No. 41, pp. 709. print.
Meeting Info.: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA. April 01-05, 2000.
ISSN: 0197-016X. Language: English.

L25 ANSWER 50 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:900194 The Genuine Article (R) Number: 364KW. Humanized anti-CEA X chimeric anti-[indium-DTPA] **bispecific antibody** (bsMAb) conjugates for radioimmunotherapy of CEA-positive tumors with Re-188.. Karacay H (Reprint); McBride W J; Sharkey R M; Griffiths G L; Spiegelman H; Hansen H; **Goldenberg D M.** GARDEN STATE CANC CTR, BELLEVILLE, NJ; IMMUNOMED INC, MORRIS PLAINS, NJ. JOURNAL OF NUCLEAR MEDICINE (MAY 2000) Vol. 41, No. 5, Supp. [S], pp. 473-473. Publisher: SOC NUCLEAR MEDICINE INC. 1850 SAMUEL MORSE DR, RESTON, VA 20190-5316. ISSN: 0161-5505. Pub. country: USA. Language: English.

L25 ANSWER 51 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:512980 Document No.: PREV200000512980. Pretargeting studies with a murine anti-colon-specific antigen-p (CSAp) X chimeric anti-(indium-DTPA) **bispecific antibody** and technetium-99m-labeled peptide.
Karacay, H. [Reprint author]; Sharkey, R. M.; McBride, W. J.; Griffiths, G. L.; Hansen, H. J.; **Goldenberg, D. M..** Garden State Cancer Center, Belleville, NJ, 07109, USA. Cancer Biotherapy and Radiopharmaceuticals, (2000) Vol. 15, No. 4, pp. 412. print.
Meeting Info.: Eighth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer. Princeton, New Jersey, USA. October 12-14, 2000.
ISSN: 1084-9785. Language: English.

L25 ANSWER 52 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:512977 Document No.: PREV200000512977. Preliminary studies on **bispecific antibody** pretargeted radioimmunotherapy against human colonic tumor xenografts in mice. Qu, Z. [Reprint author]; Hansen, H. J. [Reprint author]; Sharkey, R. M.; McBride, W. J. [Reprint author]; Losman, M. J.; Xuan, H. [Reprint author]; Ma, H. [Reprint author]; Chang, C. H. [Reprint author]; Barbet, J.; **Goldenberg, D. M.**; Leung, S. O. [Reprint author]. Immunomedics, Inc., Morris Plains, NJ, 07950, USA. Cancer Biotherapy and Radiopharmaceuticals, (2000) Vol. 15, No. 4, pp. 410. print.
Meeting Info.: Eighth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer. Princeton, New Jersey, USA. October 12-14, 2000.
ISSN: 1084-9785. Language: English.

L25 ANSWER 53 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:294910 Document No.: PREV200000294910. Pretargeting colonic tumors with engineered **bispecific antibodies** for improved radioimmunotherapy (RAIT). Leung, S. O. [Reprint author]; Qu, Z.; Sharkey, R. M.; McBride, W. J.; Losman, M. J.; Chang, C. H.; Barbet, J.; Karacay, H.; **Goldenberg, D. M.**; Hansen, H. J.. Immunomedics, Inc., Morris Plains, NJ, USA. Journal of Nuclear Medicine, (May, 2000) Vol. 41, No. 5 Suppl., pp. 270P. print.
Meeting Info.: 47th Annual Meeting of the Society of Nuclear Medicine. St. Louis, Missouri, USA. June 03-07, 2000. Society of Nuclear Medicine.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 54 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:293188 Document No.: PREV200000293188. Site-specific delivery of small

molecules to tumors via **bispecific antibody** tumor pretargeting: The use of imaging isotopes in developmental studies. McBride, W. J. [Reprint author]; **Goldenberg, D. M.**; Karacay, H.; Hansen, H. J.; Griffiths, G. L.. Immunomedics, Inc., Morris Plains, NJ, USA. Journal of Nuclear Medicine, (May, 2000) Vol. 41, No. 5 Suppl., pp. 230P. print.
Meeting Info.: 47th Annual Meeting of the Society of Nuclear Medicine. St. Louis, Missouri, USA. June 03-07, 2000. Society of Nuclear Medicine.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 55 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:291731 Document No.: PREV200000291731. Humanized anti-CEA X chimeric anti-(indium-DTPA) **bispecific antibody** (bsMAb) conjugates for radioimmunotherapy of CEA-positive tumors with 188Re. Karacay, H. [Reprint author]; McBride, W. J.; Sharkey, R. M.; Griffiths, G. L.; Spiegelman, H.; Hansen, H.; **Goldenberg, D. M.**. Garden State Cancer Center, Belleville, NJ, USA. Journal of Nuclear Medicine, (May, 2000) Vol. 41, No. 5 Suppl., pp. 120P. print.
Meeting Info.: 47th Annual Meeting of the Society of Nuclear Medicine. St. Louis, Missouri, USA. June 03-07, 2000. Society of Nuclear Medicine.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 56 OF 62 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2000117843 EMBASE Advances in the use of monoclonal antibodies in cancer radiotherapy. Govindan S.V.; **Goldenberg D.M.**; Hansen H.J.; Griffiths G.L.. S.V. Govindan, Immunomedics Inc., Corporate Headquarters, 300 American Road, Morris Plains, NJ 07950, United States. sgovindan@immunomedics.com. Pharmaceutical Science and Technology Today 3/3 (90-98) 1 Mar 2000.
Refs: 63.
ISSN: 1461-5347. CODEN: PSTTF8.
Publisher Ident.: S 1461-5347(00)00241-8. Pub. Country: United Kingdom.
Language: English. Summary Language: English.

AB The use of monoclonal antibodies (MAbs) as radiation carriers in argeted radiotherapy of cancers has produced striking clinical responses in hematologic diseases, such as non-Hodgkin's lymphoma. Novel strategies are currently being examined in an effort to improve efficacy in solid tumor therapies. Two of these strategies involve minimizing the systemic toxicity of a circulating radionuclide via 'pretargeting', and the sensitization of tumors to radiation by combination therapy with radiosensitizing drugs. Advances made in radiolabeling chemistries and in the use of alpha-particle emitters can also improve utility. Clinical evidence suggests that radioimmunotherapy may be best applied in minimal-disease and adjuvant settings in combination with other cancer therapy modalities. Copyright (C) 2000 Elsevier Science Ltd.

L25 ANSWER 57 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
1999:468597 Document No. 131:101262 Antibody/receptor targeting moiety for enhanced delivery of armed ligand. Burton, Jack; **Goldenberg, David M.** (Center for Molecular Medicine and Immunology, USA). PCT Int. Appl. WO 9936437 A1 19990722, 45 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US773 19990114. PRIORITY: US 1998-71520 19980115.

AB A method for intracellular delivery of drugs or other agents for diagnosis and therapy of malignancies or immune-mediated or inflammatory conditions. A targeting moiety of an antibody and the ligand-binding region of a selected cytokine receptor is used. The targeting moiety targets surface antigen on a specific cell population. The targeting moiety is administered to a subject, and then, after a specified interval, therapeutic or diagnostic agents linked to the cognate cytokine are given. The invention provides rapid, efficient internalization of the cytokine receptor antibody/antigen complexes. Targeting of a high-level cell surface antigen with such bispecific fusion mols. substantially increases the number of cytokine receptors over their low background level. Thus, bifunctional soluble fusion protein IL-13Rα-MN-14scFv comprising interleukin 13 receptor α chain and anti-CEA antibody was prepared and used with radiolabeled IL-13 or IL-13/onconase immunotoxin for therapy of colon, lung, breast, pancreatic, gastric, ovarian and medullary thyroid carcinoma.

L25 ANSWER 58 OF 62 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
1999:467062 The Genuine Article (R) Number: 192XV. Pretargeting studies with a humanized anti-CEA x murine anti-[In-DTPA] **bispecific antibody** construct and Tc-99m Re-188 labeled peptide.. Karacay H (Reprint); McBride W; Gautherot E; Rouvier E; Sharkey R M; Hansen H J; Barbet J; Griffiths G L; **Goldenberg D M.** IMMUNOMED INC, MORRIS PLAINS, NJ; IMMUNOTECH SA, F-13288 MARSEILLE, FRANCE; GARDEN STATE CANC CTR, BELLEVILLE, NJ. JOURNAL OF NUCLEAR MEDICINE (MAY 1999) Vol. 40, No. 5, Supp. [S], pp. 1002-1002. Publisher: SOC NUCLEAR MEDICINE INC. 1850 SAMUEL MORSE DR, RESTON, VA 20190-5316. ISSN: 0161-5505. Pub. country: USA; FRANCE. Language: English.

L25 ANSWER 59 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
1999:192953 Document No.: PREV199900192953. Studies on a humanized anti-CEA x murine anti-(In-DTPA) **bispecific antibody** construct for radioimmunotherapy of CEA-positive tumors. Karacay, H.; McBride, W.; Gautherot, E.; Rouvier, E.; Sharkey, R. M.; Hansen, H. J.; Barbet, J.; Griffiths, G. L.; **Goldenberg, D. M..** Immunomed. Inc., Morris Plains, NJ, USA. Proceedings of the American Association for Cancer Research Annual Meeting, (March, 1999) Vol. 40, pp. 644. print.
Meeting Info.: 90th Annual Meeting of the American Association for Cancer Research. Philadelphia, Pennsylvania, USA. April 10-14, 1999. American Association for Cancer Research.
ISSN: 0197-016X. Language: English.

L25 ANSWER 60 OF 62 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
1999:353722 Document No.: PREV199900353722. Pretargeting studies with a humanized anti-CEA X murine anti-(In-DTPA) **bispecific antibody** construct and Tc-99m/Re-188 labeled peptide. Karacay, H. [Reprint author]; McBride, W.; Gautherot, E.; Rouvier, E.; Sharkey, R. M.; Hansen, H. J.; Barbet, J.; Griffiths, G. L.; **Goldenberg, D. M..** Immunomedics, Inc., Morris Plains, NJ, USA. Journal of Nuclear Medicine, (May, 1999) Vol. 40, No. 5 SUPPL., pp. 225P. print.
Meeting Info.: 46th Annual Meeting of the Society of Nuclear Medicine. Los Angeles, California, USA. June 6-10, 1999. Society of Nuclear Medicine.
CODEN: JNMEAQ. ISSN: 0161-5505. Language: English.

L25 ANSWER 61 OF 62 CAPLUS COPYRIGHT 2005 ACS on STN
1998:251079 Document No. 128:304043 Specific targetting of toxins or radionuclides to tumor cells using conjugates with cytokines and **bispecific antibodies** recognizing a cell surface marker and the cytokine. **Goldenberg, David M.** (Immunomedics, Inc., USA). PCT Int. Appl. WO 9816254 A1 19980423, 26 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN,

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     TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1997-US17924
     19971014.  PRIORITY: US 1996-28430 19961017.
AB   Methods for treatment of B-cell malignancies that make use of slowly
     internalizing surface antigens and that increase the stability of binding
     of antibodies to the surfaces of lymphomas are described.  This is
     achieved using conjugates of toxins or radionuclides with interleukin 15
     in combination with bispecific antibodies that
     recognize the α-chain of interleukin 15 and a malignant cell marker.
     The interleukin 15 receptor can be bridged to a slowly internalizing
     surface antigens using the bispecific antibody to
     introduce cytotoxins into cells bearing the antigen and the receptor.

L25  ANSWER 62 OF 62  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO
     9604313 A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG,
     BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG,
     KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO,
     RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI,
     CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE,
     SN, TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491
     19950801.  PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a tumor or
     infectious agent are used to overcom the multidrug resistant phenotype.
     These immunoconjugates and composites also can be used diagnostically to
     determine whether the failure of traditional chemotherapy is due to the
     presence of multidrug resistant tumor cells, multidrug resistant
     HIV-infected cells or multidrug resistant infectious agents.  In example,
     polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin,
     dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer
     treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
     targeted to multidrug resistant Pseudomonas aeruginosa were also prepared


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 NEWS  6  DEC 01  LISA now available on STN
 NEWS  7  DEC 09  12 databases to be removed from STN on December 31, 2004
 NEWS  8  DEC 15  MEDLINE update schedule for December 2004
 NEWS  9  DEC 17  ELCOM reloaded; updating to resume; current-awareness
                  alerts (SDIs) affected
 NEWS  10 DEC 17  COMPUAB reloaded; updating to resume; current-awareness
                  alerts (SDIs) affected
 NEWS  11 DEC 17  SOLIDSTATE reloaded; updating to resume; current-awareness
                  alerts (SDIs) affected
 NEWS  12 DEC 17  CERAB reloaded; updating to resume; current-awareness
                  alerts (SDIs) affected
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 NEWS  15 DEC 30  CAPLUS - PATENT COVERAGE EXPANDED
 NEWS  16 JAN 03  No connect-hour charges in EPFULL during January and
                  February 2005
 NEWS  17 JAN 11  CA/CAPLUS - Expanded patent coverage to include Russia
                  (Federal Institute of Industrial Property)

 NEWS EXPRESS  JANUARY 10 CURRENT WINDOWS VERSION IS V7.01a, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 10 JANUARY 2005

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=> file medline embase biosis scisearch caplus
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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

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=> s multidrug transporter protein
L1          109 MULTIDRUG TRANSPORTER PROTEIN

=> s l1 and P glycoprotein
L2           61 L1 AND P GLYCOPROTEIN

=> s l2 and "OtrB"
L3            2 L2 AND "OTRB"

=> dup remove l3
PROCESSING COMPLETED FOR L3
L4            2 DUP REMOVE L3 (0 DUPLICATES REMOVED)

=> d l4 1-2 cbib abs

L4   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
AB   Immunoconjugates of a diagnostic agent and an antibody component that
     binds an epitope of a multidrug transporter
     protein are disclosed.  Thesse immunoconjugates are used in in
     vivo diagnostic methods to determine whether the failure of traditional
     chemotherapy is due to the presence of multidrug resistant tumor cells,
     multidrug resistant HIV-infected cells or multidrug resistant infectious
     agents.

L4   ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen
     associated with a tumor or infectious agent are used to overcom the multidrug
     resistant phenotype.  These immunoconjugates and composites also can be
     used diagnostically to determine whether the failure of traditional
     chemotherapy is due to the presence of multidrug resistant tumor cells,
     multidrug resistant HIV-infected cells or multidrug resistant infectious
     agents.  In example, polyspecific immunoconjugates containing G-CSF,
     90Yttrium, doxorubicin, dextran and anti-P-glycoprotein
     /anti-carcinoembryonic antigen bispecific antibody were prepared for colon
     cancer treatment.  99mTc- and 111Indium-labeled polyspecific
     immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa
     were also prepared


=> d his

     (FILE 'HOME' ENTERED AT 17:21:20 ON 24 JAN 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 17:21:31 ON
     24 JAN 2005
L1          109 S MULTIDRUG TRANSPORTER PROTEIN
L2           61 S L1 AND P GLYCOPROTEIN
L3            2 S L2 AND "OTRB"
L4            2 DUP REMOVE L3 (0 DUPLICATES REMOVED)

=> s l1 and transporter gene
L5            3 L1 AND TRANSPORTER GENE

=> dup remove l5
PROCESSING COMPLETED FOR L5
L6            3 DUP REMOVE L5 (0 DUPLICATES REMOVED)

=> d l6 1-3 cbib abs

L6   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
2001:464796  Document No. 135:192716  Activity of the Kluyveromyces lactis
     Pdr5 multidrug transporter is modulated by the Sit4 protein phosphatase.
     Chen, Xin Jie (Molecular Genetics and Evolution Group, Research School of
     Biological Sciences, The Australian National University, Canberra, 2601,
     Australia).  Journal of Bacteriology, 183(13), 3939-3948 (English) 2001.
     CODEN: JOBAAY.  ISSN: 0021-9193.  Publisher: American Society for
     Microbiology.
AB   A possible role for posttranslational modifications in regulating the
     activity of ATP-binding cassette (ABC) transporters has not been well
     established.  In this study, the drug efflux ABC transporter
     gene KlPDR5 was isolated from the budding yeast Kluyveromyces
     lactis, and it was found that the encoded KlPdr5 drug pump is
     posttranslationally regulated by the type 2A-related Ser/Thr protein
     phosphatase, Sit4p.  The KlPdr5 transporter is a protein of 1,525 amino
     acids sharing 63.8% sequence identity with its Saccharomyces cerevisiae
     counterpart, ScPdr5p.  Overexpression of the KlPDR5 gene confers
     resistance to oligomycin, antimycin, econazole, and ketoconazole, whereas
     cells with a disrupted allele of KlPDR5 are hypersensitive to the drugs
     and have a decreased capacity to carry out efflux of the anionic
     fluorescent dye rhodamine 123.  It was found that a chromosomal disruption
     of KlPDR5 abolishes the drug-resistant phenotype associated with sit4
     mutations and that a synergistic hyperresistance to the drugs can be
     created by overexpressing KlPDR5 in sit4 mutants.  These data strongly
     indicate that the multidrug-resistant phenotype of sit4 mutants is
     mediated by neg. modulating the activity of KlPdr5p.  As the
     transcriptional level of KlPDR5 and the steady-state level of KlPdr5p are
     not significantly affected by mutations in SIT4, the regulation by Sit4p
     appears to be a posttranslational process.

L6   ANSWER 2 OF 3  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation.  on STN
1997:283676  Document No.: PREV199799582879. Bmr3, a third multidrug
     transporter gene of Bacillus subtilis. Ohki, R.; Murata,
     M.. Univ. Kyorin, Tokyo, Japan. Abstracts of the General Meeting of the
     American Society for Microbiology, (1997) Vol. 97, No. 0, pp. 366.
     Meeting Info.: 97th General Meeting of the American Society for
     Microbiology. Miami Beach, Florida, USA. May 4-8, 1997.
     ISSN: 1060-2011. Language: English.

L6   ANSWER 3 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
1995:689332  Document No. 123:277827  Two highly similar multidrug
     transporters of Bacillus subtilis whose expression is differentially
     regulated.  Ahmed, Maqbool; Lyass, Ljuba; Markham, Penelope N.; Taylor,
     Steven S.; Vazquez-Laslop, Nora; Neyfakh, Alexander A. (Dep. Med. Chem.
     Pharmacognosy, Univ. Illinois, Chicago, IL, 60612, USA).  Journal of
     Bacteriology, 177(14), 3904-10 (English) 1995.  CODEN: JOBAAY.  ISSN:
     0021-9193.  Publisher: American Society for Microbiology.
AB   The Bacillus subtilis genome encodes two multidrug efflux transporters
     sharing 51% sequence identity: Bmr, described previously, and Blt,
     described here.  Overexpression of either transporter in B. subtilis leads
     to a similar increase in resistance to ethidium bromide, rhodamine and
     acridine dyes, tetraphenylphosphonium, doxorubicin, and fluoroquinolone
     antibiotics.  However, Blt differs widely from Bmr in its expression
     pattern.  Under standard cultivation conditions, B. subtilis expresses Bmr but
     Blt expression in undetectable.  We have previously shown that Bmr
     expression is regulated by BmrR, a member of the family of MerR-like
     transcriptional activators.  Here we show that blt transcription is
     regulated by another member of the same family, BltR.  The DNA-binding
     domains of BmrR and BltR are related, but their putative inducer-binding
     domains are dissimilar, suggesting that Bmr and Blt are expressed in
     response to different inducers.  Indeed, rhodamine, a substrate of Bmr and
     Blt and a known inducer of Bmr expression, does not induce Blt expression.
     Blt expression has been observed only in B. subtilis carrying mutation acfA,
     which, as we show here, alters the sequence of the blt gene promoter.
     Unlike bmr, which is transcribed as a monocistronic mRNA, blt is
     cotranscribed with a downstream gene encoding a putative
     acetyltransferase.  Overall, the differences in transcriptional control
     and operon organization between bmr and blt suggest that the transporters
     encoded by these genes have independent functions involving the transport
     of distinct physiol. compds.


=> s l1 and "Tel(L)"
L7            2 L1 AND "TEL(L)"

=> dup remove l7
PROCESSING COMPLETED FOR L7
L8            2 DUP REMOVE L7 (0 DUPLICATES REMOVED)

=> d l8 1-2 cbib abs

L8   ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017  Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
AB   Immunoconjugates of a diagnostic agent and an antibody component that
     binds an epitope of a multidrug transporter
     protein are disclosed.  Thesse immunoconjugates are used in in
     vivo diagnostic methods to determine whether the failure of traditional
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          chemotherapy is due to the presence of multidrug resistant tumor cells,
          multidrug resistant HIV-infected cells or multidrug resistant infectious
          agents.

L8   ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
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          A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
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          KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
          SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
          DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
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AB   Polyspecific immunoconjugates and antibody composites that bind a
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          multidrug resistant HIV-infected cells or multidrug resistant infectious
          agents.  In example, polyspecific immunoconjugates containing G-CSF,
          90Yttrium, doxorubicin, dextran and anti-P-glycoprotein/anti-
          carcinoembryonic antigen bispecific antibody were prepared for colon cancer
          treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
          targeted to multidrug resistant Pseudomonas aeruginosa were also prepared


=> s l1 and "Mmr"
L9            2 L1 AND "MMR"

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10            2 DUP REMOVE L9 (0 DUPLICATES REMOVED)

=> d l10 1-2 cbib abs

L10  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug resistance in
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          M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
          40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
          CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
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          TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
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          CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
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          protein are disclosed.  Thesse immunoconjugates are used in in
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          agents.

L10  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
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          Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
          A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
          CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
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     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
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     carcinoembryonic antigen bispecific antibody were prepared for colon cancer
     treatment.  99mTc- and 111Indium-labeled polyspecific immunoconjugate
     targeted to multidrug resistant Pseudomonas aeruginosa were also prepared


=> s transporter
L11     196610 TRANSPORTER

=> s l11 and "P glycoprotein"
L12      10709 L11 AND "P GLYCOPROTEIN"

=> s l12 and "OtrB"
L13          2 L12 AND "OTRB"

=> dup remove l13
PROCESSING COMPLETED FOR L13
L14           2 DUP REMOVE L13 (0 DUPLICATES REMOVED)

=> d l14 1-2 cbib abs

L14  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
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     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
AB   Immunoconjugates of a diagnostic agent and an antibody component that
     binds an epitope of a multidrug transporter protein are
     disclosed.  Thesse immunoconjugates are used in in vivo diagnostic methods
     to determine whether the failure of traditional chemotherapy is due to the
     presence of multidrug resistant tumor cells, multidrug resistant
     HIV-infected cells or multidrug resistant infectious agents.

L14  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
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```
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein
     /anti-carcinoembryonic antigen bispecific antibody were prepared for colon
     cancer treatment.  99mTc- and 111Indium-labeled polyspecific
     immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa
     were also prepared


=> s l11 and "OtrB"
L15            2 L11 AND "OTRB"

=> dup remove l15
PROCESSING COMPLETED FOR L15
L16            2 DUP REMOVE L15 (0 DUPLICATES REMOVED)

=> d l16 1-2 cbib abs

L16  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017  Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
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     HIV-infected cells or multidrug resistant infectious agents.

L16  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658  Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
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     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared


=> s l11 and "Tel(L)"
L17          2 L11 AND "TEL(L)"

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PROCESSING COMPLETED FOR L17
L18           2 DUP REMOVE L17 (0 DUPLICATES REMOVED)

=> d l18 1-2 cbib abs

L18  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
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     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
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     HIV-infected cells or multidrug resistant infectious agents.

L18  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
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     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared


=> s l11 and "Mmr"
L19         14 L11 AND "MMR"

=> dup remove l19
PROCESSING COMPLETED FOR L19
L20          10 DUP REMOVE L19 (4 DUPLICATES REMOVED)
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=> d l20 1-10 cbib abs

L20  ANSWER 1 OF 10  CAPLUS  COPYRIGHT 2005 ACS on STN
2003:556633  Document No. 139:290771  Differential HLA Gene Expression in
     Measles Vaccine Seropositive and Seronegative Subjects: A Pilot Study.
     Dhiman, Neelam; Bonilla, Ruben G.; Jacobson, Robert M.; O'Kane, Dennis;
     Poland, Gregory A. (Mayo Vaccine Research Group, Mayo, Rochester, MN,
     55905, USA). Scandinavian Journal of Infectious Diseases, 35(5), 332-336
     (English) 2003.  CODEN: SJIDB7.  ISSN: 0036-5548.  Publisher: Taylor &
     Francis Ltd..
AB   This is the first study using GeneChip technol. to elucidate genetic
     determinants of the measles vaccine response.  A comparative gene
     expression study was conducted using Affymetrix's Human GeneChip U-95A in
     5 human subjects immunized with a 'booster' dose of measles vaccine
     (Attenuax, Merck) to determine whether serol. distinct subjects exhibit
     differential expression of human leukocyte antigen (HLA) genes.  Healthy
     individuals aged 15-25 yr, previously immunized with 2 doses of
     measles-mumps-rubella-II (MMR-II) vaccine, were classified as
     measles vaccine IgG-specific antibody seronegatives (n=2) and
     seropositives (n=3).  Changes in expression of HLA genes in seronegatives
     and seropositives were studied on days 7 and 14 post-measles vaccination
     using Microarray Suite 5.0 (MAS 5.0).  There was increased expression of
     the HLA class I-B, HLA class II cluster of DMA, DMB, TAP1, TAP2 and HLA-DR
     genes, and decreased expression of HLA class I MICB mol., HLA class I-A
     and major histocompatibility complex class III HSP 70 genes on day 7 or
     day 14 postvaccination in seropositives compared with seronegatives.
     These results suggest an association between antibody response and
     differential HLA gene activation and may explain one potential mechanism
     underlying measles vaccine non-response.

L20  ANSWER 2 OF 10     MEDLINE on STN                     DUPLICATE 1
2003261599.     PubMed ID: 12786997.   Lack of association between
     transporter associated with antigen processing (TAP) and HLA-DM
     gene polymorphisms and antibody levels following measles vaccination.
     Dhiman N; Ovsyannikova I G; Pinsky N A; Vierkant R A; Jacobsen S J;
     Jacobson R M; Poland G A. (Mayo Vaccine Research Group, Mayo Clinic and
     Foundation, 611C Guggenheim Building, 200 First Street SW, Rochester, MN
     55905, USA. ) European journal of immunogenetics : official journal of the
     British Society for Histocompatibility and Immunogenetics, (2003 Jun) 30
     (3) 195-200.  Journal code: 9106962. ISSN: 0960-7420. Pub. country:
     England: United Kingdom. Language: English.
AB   The transporter associated with antigen processing (TAP) and
     human leukocyte antigen-DM (HLA-DM) genes are involved in the
     antigen-processing pathway of both HLA class I and class II-restricted
     antigen presentation.  We hypothesized that polymorphisms within the TAP
     and DM genes may influence antibody levels following measles vaccination.
     We examined TAP and DM polymorphisms in 242 school children from Olmsted
     County, Minnesota, USA who received one dose of measles-mumps-rubella-II (
     MMR-II) vaccine at the age of 15 months.  Based on the level of
     serum measles-specific immunoglobulin G (IgG) antibodies, subjects were
     classified as seronegatives (n = 72) or seropositives (n = 170).  We
     determined TAP1 and TAP2 allele types by polymerase chain reaction (PCR)
     amplification of specific alleles (PASA) and determined DM allele type by
     PCR amplification followed by direct sequencing of the polymorphic sites.
     We analysed the data for any TAP or DM allelic association with antibody
     levels post measles vaccination using the chi-square test and univariate
     linear regression analysis.  We found no trend in the overall distribution
     of TAP and DM genotype frequencies between seronegative and seropositive
     subjects, suggesting that TAP and DM polymorphism and antibody levels
     following measles vaccination are not directly associated.  In addition,
     we did not find an association between TAP (TAP1, P = 0.71; TAP2, P =
     0.87) or DM (DMA, P = 0.42; DMB, P = 0.71) homozygosity and seronegativity
     to measles vaccine in this study group.  Our study suggests that TAP and
     DM gene polymorphisms do not influence antibody levels post measles
     vaccination.
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L20  ANSWER 3 OF 10  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2004295079  EMBASE  The neurobiology of autism: New pieces of the puzzle.
     Acosta M.T.; Pearl P.L.. Dr. M.T. Acosta, Department of Neurology,
     Children's National Medical Center, 111 Michigan Avenue NW, Washington, DC
     20010-2970, United States. macosta@cnmc.org. Current Neurology and
     Neuroscience Reports  3/2 (149-156)  2003.
     Refs: 102.
     ISSN: 1528-4042. Pub. Country: United Kingdom. Language: English. Summary
     Language: English.
AB   The neurobiologic basis of autism is reviewed, with discussion of evidence
     from genetic, magnetic resonance imaging, neuropathology, and functional
     neuroimaging studies. Although autism is a behaviorally valid syndrome, it
     is remarkably heterogeneous and involves multiple developmental domains as
     well as a wide range of cognitive, language, and socioemotional
     functioning. Although multiple etiologies are implicated, recent advances
     have identified common themes in pathophysiology. Genetic factors play a
     primary role, based on evidence from family studies, identification of
     putative genes using genome-wide linkage analyses, and comorbidities with
     known genetic mutations. The RELN gene, which codes for an extracellular
     protein guiding neuronal migration, has been implicated in autism.
     Numerous neuropathologic changes have been described, including
     macroencephaly, acceleration and then deceleration in brain growth,
     increased neuronal packing and decreased cell size in the limbic system,
     and decreased Purkinje cell number in the cerebellum. Abnormalities in
     organization of the cortical minicolumn, representing the fundamental
     subunit of vertical cortical organization, may underlie the pathology of
     autism and result in altered thalamocortical connections, cortical
     disinhibition, and dysfunction of the arousal-modulating system of the
     brain. The role of acquired factors is speculative, with insufficient
     evidence to link the measles-mumps-rubella (MMR) vaccine with
     autism or to change immunization practices. Copyright .COPYRGT. 2003 by
     Current Science Inc.

L20  ANSWER 4 OF 10  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2002413206  EMBASE  Vanadate inhibits the ATPase activity and DNA binding
     capability of bacterial MutS. A structural model for the vanadate-MutS
     interaction at the Walker A motif. Pezza R.J.; Villarreal M.A.; Montich
     G.G.; Argarana C.E.. C.E. Argarana, Ctro. Investig. Quim. Biol. Cordoba,
     UNC-CONICET, Departamento de Quimica Biologica, Cordoba, Argentina.
     carga@dqb.fcq.unc.edu.ar. Nucleic Acids Research  30/21 (4700-4708)  1 Nov
     2002.
     Refs: 50.
     ISSN: 0305-1048. CODEN: NARHAD. Pub. Country: United Kingdom. Language:
     English. Summary Language: English.
AB   MutS, a member of the ABC ATPases superfamily, is a mismatch DNA-binding
     protein constituent of the DNA post-replicative mismatch repair system (
     MMRS). In this work, it is shown that the ATPase activity of
     Pseudomonas aeruginosa and Escherichia coli MutS is inhibited by ortho-
     and decavanadate. Structural comparison of the region involved in the ATP
     binding of E.coli MutS with the corresponding region of other ABC ATPases
     inhibited by vanadate, including the myosin-orthovanadate-Mg complex,
     showed that they are highly similar. From these results it is proposed
     that the orthovanadate inhibition of MutS ATPase can take place by a
     similar mechanism to that described for other ATPases. Docking of
     decavanadate on the ATP-binding region of MutS showed that the
     energetically more favorable interaction of this compound would take place
     with the complex MutS-ADP-Mg, suggesting that the inhibitory effect could
     be produced by a steric impediment of the protein ATP/ADP exchange.
     Besides the effect observed on the ATPase activity, vanadate also affects
     the DNA-binding capability of the protein, and partially inhibits the
     oligomerization of MutS and the temperature-induced inactivation of the
     protein. From the results obtained, and considering that vanadate is an
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intracellular trace component, this compound could be considered as a new modulator of the **MMRS**.

L20 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN

2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L20 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN

1999:734683 Document No. 132:216310 Resistance to macrolides mediated by efflux mechanisms. Sutcliffe, Joyce (Department of Infectious Diseases, Pfizer Central Research Inc, Groton, CT, 06340, USA). Current Opinion in Anti-Infective Investigational Drugs, 1(4), 403-412 (English) 1999. CODEN: COADFY. ISSN: 1464-8458. Publisher: Current Drugs Ltd..

AB A review with 92 refs. Drug transport proteins can be divided into two classes: secondary **transporters**, which couple extrusion of drugs with ion exchange, and ATP-binding cassette (ABC) **transporters**, which utilize the energy of a phosphate bond upon ATP hydrolysis to efflux drugs. Efflux is mediated by either a pump with narrow specificity, e.g, Mef and MsrA, or a pump with broad specificity, e.g., AcrAB-TolC, AcrEF-TolC, MexAB-OprM, MexCD-OprJ, AmrAB-OprA, MtrCDE, EmrE, MdfA and Cmr. Staphylococcus epidermidis harbors a plasmid with an msrA determinant, an ABC-type **transporter** specific for 14- and 15-membered macrolides and streptogramin B. An almost identical gene resides in Staphylococcus aureus (S aureus), although it appears to be uncommon. MsrA appears to be the ATP binding component of the ABC **transporter** and likely works in concert with various pumps that have been identified on plasmids or on the chromosome of staphylococcal strains. MsrB, found in Staphylococcus xylosus, is identical to the C-terminal portion of MsrA and likely functions as a dimer. MefA and MefE, initially described in Streptococcus pyogenes and Streptococcus pneumoniae, resp., have been folded into a single family, MefA, because the two genes are approx. 90% identical at the amino acid level. The MefA protein is a member of the major facilitator superfamily (MFS) and can efflux 14- and 15- membered macrolides. Resistance to 16-membered macrolides is not seen even in the presence of inducing concns. of erythromycin. Strains harboring the mefA gene remain susceptible to streptogramin B and clindamycin. Interestingly, the MefA protein conferred macrolide resistance when cloned into Escherichia coli (E coli). More recently, mefA has been described in many different streptococcal species, moderately erythromycin-resistant strains of Enterococcus faecium, Micrococcus luteus and Corynebacterium jeikeium. Other members of the MFS class that can cause the active egress of erythromycin include MdfA in E coli and Cmr in Corynebacterium glutamicum. The latter two require overexpression to confer approx. 4-fold level of resistance. Another putative pump, MreA, was described in a clin. strain of Streptococcus agalactiae, but recent work demonstrates that this protein likely encodes a riboflavin kinase (ribC). The AcrAB-TolC pump is found in E coli and similar members of the resistance-nodulation-cell division family appear to be present in Haemophilus influenzae (AcrAB-like), Pseudomonas aeruginosa (MexAB-OprM, MexCD-OprJ), Burkholderia pseudomallei (AmrAB-OprA) and Neisseria gonorrhoeae (MtrCDE). The **transporters** in the Gram-neg. bacteria (AcrB, AcrF, MexB, MexD, AmrB, MtrD) work in concert with an accessory protein (AcrA, AcrE, MexA, MexC, AmrA, MtrC) which spans the periplasmic space and interacts with a porin in the outer membrane (TolC, TolC(), OprM, OprJ, OprA, MtrE). AcrAB-TolC and MtrCDE confer resistance to hydrophobic antibiotics (including erythromycin), detergents and dyes, while the two multidrug pumps from Pseudomonas overlap in their ability to efflux compds. such as tetracycline, novobiocin, chloramphenicol, erythromycin and fluoroquinolones, but show specificity toward certain β-lactams and fourth generation cephalosporins. AmrAB-OprA confers high-level resistance to aminoglycosides and macrolides. Some of these pumps are constitutive, e.g., AcrAB-TolC, AcrAB-Omp2, MexAB-OprM, AmrAB-OprA, while others remain silent until promoter or regulatory mutations allow their expression. Macrolide resistance can also be conferred by members of the small multidrug resistance family, EmrE and **Mmr**. EmrE is chromosomally encoded in E coli and has no known transmembrane linker or outer membrane channel associated with it. Reconstituted EmrE catalyzes the efflux or erythromycin and tetracycline, in addition to lipophilic monovalent cations. **Mmr** has been cloned from Mycobacterium tuberculosis and confers erythromycin resistance in Mycobacterium smegmatis. All the resistance determinants are intrinsic and chromosomally encoded, with the exception of msrA and mefA. Recently, mefA was transferred to naive strains by conjugation; it is likely mobile by a chromosomally integrated transposon. MsrA is generally plasmid-mediated. The expression of both genes is inducible by erythromycin.

L20 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN

1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US9491 19950801. PRIORITY: US 1994-286430 19940805.

AB Polyspecific immunoconjugates and antibody composites that bind a multidrug **transporter** protein and an antigen associated with a tumor or infectious agent are used to overcom the multidrug resistant phenotype. These immunoconjugates and composites also can be used diagnostically to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents. In example, polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen bispecific antibody were prepared for colon cancer treatment. 99mTc- and 111Indium-labeled polyspecific immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa were also prepared

L20 ANSWER 8 OF 10 MEDLINE on STN

93316854. PubMed ID: 8326867. Streptomyces antibioticus contains at least three oleandomycin-resistance determinants, one of which shows similarity with proteins of the ABC-**transporter** superfamily. Rodriguez A M; Olano C; Vilches C; Mendez C; Salas J A. (Departamento de Biologia Funcional, Universidad de Oviedo, Spain.) Molecular microbiology, (1993 May) 8 (3) 571-82. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Three different DNA fragments of an oleandomycin producer, Streptomyces antibioticus, conferring oleandomycin resistance were cloned in plasmid pIJ702 and expressed in Streptomyces lividans and in Streptomyces albus. These oleandomycin resistance determinants were designated as oleA (pOR400), oleB (pOR501) and oleC (pOR800). oleA and oleC are closely linked in the chromosome as they were both obtained together in two cosmid clones that were isolated from a genomic library. Sequencing of the oleC resistance determinant revealed four complete open reading frames (ORFs) and the C-terminal end of a fifth. The functions of orf1 and orf2 are unknown since they did not show significant similarity with other sequences in the data bases. The orf3 gene product has similarity with some proteins involved in iron and vitamin B12 uptake in bacteria. The orf4 gene product had a hydrophilic profile and showed important similarity with proteins containing typical ATP-binding domains characteristic of the ABC-**transporter** superfamily and involved in membrane transport and, particularly, with several genes conferring resistance to various macrolide antibiotics and anticancer drugs. The last gene, orf5, is translationally coupled to orf4 and codes for a hydrophobic polypeptide containing several transmembrane domains characteristic of integral membrane proteins. Subcloning and deletion experiments limited the resistance determinant to a 0.9 kb PstI-SphI fragment and only orf4 is included in this fragment. These results suggest that resistance to oleandomycin conferred by oleC (orf4) is probably due to an efflux transport system of the ABC-**transporter** superfamily.

L20 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
1993:642306 Document No. 119:242306 Molecular cloning and characterization of two lincomycin-resistance genes, lmrA and lmrB from Streptomyces lincolnensis 78-11. Zhang, Hui Zhan; Schmidt, Heike; Piepersberg, Wolfgang (Berg. Univ. GH Wuppertal, Wuppertal, W-5600/1, Germany). Molecular Microbiology, 6(15), 2147-57 (English) 1992. CODEN: MOMIEE. ISSN: 0950-382X.

AB Two different lincomycin-resistance determinants (lmrA and lmB) from S. linocolnensis 78-11 were cloned in Streptomyces lividans 66 TK23. The gene lmrA was localized on a 2.16 kb fragment, the determined nucleotide sequence of which encoded a single open reading frame 1446 bp long. Anal. of the deduced amino acid sequence suggested the presence of 12 membrane-spanning domains and showed significant similarities to the methylenomycin-resistance protein (**Mmr**) from S. coelicolor, the QacA protein from Staphylococcus aureus, and several tetracycline-resistance proteins from both Gram-pos. and Gram-neg. bacteria, as well as to some sugar-transport proteins from Escherichia coli. The lmrB gene was actively expressed from a 2.7 kb fragment. An open reading frame of 837 bp could be localized which encoded a protein that was significantly similar to 23 S rRNA adenine(2058)-N-methyltransferases conferring macrolide-lincosamide-streptogramin resistance. LmrB also had putative rRNA methyltransferase activity since lincomycin resistance of ribosomes was induced in lmrB-containing strains. Surprisingly, both enzymes, LmrA and LmrB, had a substrate specificity restricted to lincomycin and did not cause resistance to other lincosamides such as celesticetin and clindamycin, or to macrolides.

L20 ANSWER 10 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
1991:442803 Document No. 115:42803 Efflux-mediated antiseptic resistance gene qacA from Staphylococcus aureus: common ancestry with tetracycline- and sugar-transport proteins. Rouch, D. A.; Cram, D. S.; DiBerardino, D.; Littlejohn, T. G.; Skurray, R. A. (Dep. Microbiol., Monash Univ., Clayton, 3168, Australia). Molecular Microbiology, 4(12), 2051-62 (English) 1990. CODEN: MOMIEE. ISSN: 0950-382X.

AB Resistance to intercalating dyes (ethidium, acriflavine) and other organic cations, such as quaternary ammonium-type antiseptic compds., mediated by the S. aureus plasmid pSK1 is specified by an energy-dependent export mechanism encoded by the qacA gene. From nucleotide sequence anal., qacA is predicted to encode a protein of Mr 55,017 containing 514 amino acids. The gene is likely to initiate with a CUG codon, and a 36 bp palindrome immediately preceding qacA, along with an upstream reading frame ORF188 with homol. to the TetR repressors, may be components of a regulatory circuit. The putative polypeptide specified by qacA has properties typical of a cytoplasmic membrane protein, and is indicated to be a member

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     of a transport protein family that includes proteins responsible for
     export-mediated resistance to tetracycline and methylenomycin, and uptake
     of sugars and quinate.  The anal. suggests that N- and C-terminal regions
     of these proteins are involved in energy coupling (proton translocation)
     and substrate transport, resp.  The last common ancestor of the qacA and
     related tet (tetracycline resistance) lineages is inferred to have been
     repressor controlled, as occurs for modern tet determinants from
     gram-neg., but not those from gram-pos., bacteria.


=> s l11 and "AtcII"
L21           0 L11 AND "ATCII"

=> s lll and
MISSING TERM AFTER LLL AND
Operators must be followed by a search term, L-number, or query name.

=> s l11 and "ActII"
L22           9 L11 AND "ACTII"

=> dup remove l22
PROCESSING COMPLETED FOR L22
L23            5 DUP REMOVE L22 (4 DUPLICATES REMOVED)

=> d l23 1-5 cbib abs

L23  ANSWER 1 OF 5  CAPLUS  COPYRIGHT 2005 ACS on STN
2002:778169  Document No. 137:290025  Genes of calicheamicin biosynthesis and
     resistance of Micromonospora echinospora and their uses.  Thorson, Jon
     (Memorial Sloan-Kettering Cancer Center, USA).  PCT Int. Appl. WO
     2002079465 A2 20021010, 179 pp.  DESIGNATED STATES: W:  AE, AG, AL, AM,
     AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK,
     DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,
     KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
     MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG,
     CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR,
     NE, NL, PT, SE, SN, TD, TG, TR.  (English).  CODEN: PIXXD2.  APPLICATION:
     WO 2001-US44285 20011128.  PRIORITY: US 2000-724797 20001128.
AB   An isolated gene cluster of Micromonospora echinospora which codes for
     calicheamicin biosynthesis is identified and characterized.  The
     biosynthetic gene cluster contains genes encoding proteins and enzymes
     used in the biosynthetic production of calicheamicin, including the
     aryltetrasaccharide and aglycon.  The gene cluster also includes the gene
     coding for the protein conferring calicheamicin resistance.  The invention
     also provides isolated genes of the biosynthetic cluster and their
     corresponding proteins.  In addition, the invention relates to DNA
     hybridizing with the calicheamicin gene cluster and the isolated genes of
     that cluster.  Expression vectors containing genes of the biosynthetic gene
     and their functional variants are also provided.  The invention also
     relates to host cells conjugated with DNA isolated from the Micromonospora
     echinospora spp. calichensis genome.

L23  ANSWER 2 OF 5  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017  Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
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1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L23 ANSWER 3 OF 5 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

1999034296 EMBASE The mithramycin gene cluster of Streptomyces argillaceus contains a positive regulatory gene and two repeated DNA sequences that are located at both ends of the cluster. Lombo F.; Brana A.F.; Mendez C.; Salas J.A.. J.A. Salas, Departamento de Biologia Funcional, I.U.B.A.-C.S.I.C., Universidad de Oviedo, 33006 Oviedo, Spain. Jasf@sauron.quimica.uniovi.es. Journal of Bacteriology 181/2 (642-647) 1999.
Refs: 41.
ISSN: 0021-9193. CODEN: JOBAAY. Pub. Country: United States. Language: English. Summary Language: English.

AB Sequencing of a 4.3-kb DNA region from the chromosome of Streptomyces argillaceus, a mithramycin producer, revealed the presence of two open reading frames (ORFs). The first one (orfA) codes for a protein that resembles several transport proteins. The second one (mtmR) codes for a protein similar to positive regulators involved in antibiotic biosynthesis (DnrI, SnoA, **ActII**-orf4, CcaR, and RedD) belonging to the Streptomyces antibiotic regulatory protein (SARP) family. Both ORFs are separated by a 1.9-kb, apparently noncoding region. Replacement of the mtmR region by an antibiotic resistance cassette completely abolished mithramycin biosynthesis. Expression of mtmR in a high-copy-number vector in S. argillaceus caused a 16-fold increase in mithramycin production. The mtmR gene restored actinorhodin production in Streptomyces coelicolor JF1 mutant, in which the actinorhodin-specific activator **ActII**-orf4 is inactive, and also stimulated actinorhodin production by Streptomyces lividans TK21. A 241-bp region located 1.9 kb upstream of mtmR was found to be repeated approximately 50 kb downstream of mtmR at the other end of the mithramycin gene cluster. A model to explain a possible route for the acquisition of the mithramycin gene cluster by S. argillaceus is proposed.

L23 ANSWER 4 OF 5 MEDLINE on STN DUPLICATE 1

1998361886. PubMed ID: 9696745. A silent ABC **transporter** isolated from Streptomyces rochei F20 induces multidrug resistance. Fernandez-Moreno M A; Carbo L; Cuesta T; Vallin C; Malpartida F. (Centro Nacional de Biotecnologia, CSIC, Campus Universidad Autonoma de Madrid, Cantoblanco 28049, Madrid, Spain.) Journal of bacteriology, (1998 Aug) 180 (16) 4017-23. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB In the search for heterologous activators for actinorhodin production in Streptomyces lividans, 3.4 kb of DNA from Streptomyces rochei F20 (a streptothricin producer) were characterized. Subcloning experiments showed that the minimal DNA fragment required for activation was 0.4 kb in size. The activation is mediated by increasing the levels of transcription of the **actII**-ORF4 gene. Sequencing of the minimal activating fragment did not reveal any clues about its mechanism; nevertheless, it was shown to overlap the 3' end of two convergent genes, one of whose translated products (ORF2) strongly resembles that of other genes belonging to the ABC **transporter** superfamily.
Computer-assisted analysis of the 3.4-kb DNA sequence showed the 3' terminus of an open reading frame (ORF), i.e., ORFA, and three complete ORFs (ORF1, ORF2, and ORFB). Searches in the databases with their respective gene products revealed similarities for ORF1 and ORF2 with ATP-binding proteins and transmembrane proteins, respectively, which are found in members of the ABC **transporter** superfamily. No similarities for ORFA and ORFB were found in the databases. Insertional inactivation of ORF1 and ORF2, their transcription analysis, and their

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          cloning in heterologous hosts suggested that these genes were not
          expressed under our experimental conditions; however, cloning of ORF1 and
          ORF2 together (but not separately) under the control of an expressing
          promoter induced resistance to several chemically different drugs:
          oleandomycin, erythromycin, spiramycin, doxorubicin, and tetracycline.
          Thus, this genetic system, named msr, is a new bacterial multidrug ABC
          transporter.

L23  ANSWER 5 OF 5  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
          antibody composites for targeting the multidrug resistant phenotype.
          Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
          A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
          CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
          KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
          SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
          DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
          TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
          PRIORITY: US 1994-286430 19940805.
AB        Polyspecific immunoconjugates and antibody composites that bind a
          multidrug transporter protein and an antigen associated with a
          tumor or infectious agent are used to overcom the multidrug resistant
          phenotype.  These immunoconjugates and composites also can be used
          diagnostically to determine whether the failure of traditional chemotherapy is
          due to the presence of multidrug resistant tumor cells, multidrug
          resistant HIV-infected cells or multidrug resistant infectious agents.  In
          example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
          doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
          bispecific antibody were prepared for colon cancer treatment.  99mTc- and
          111Indium-labeled polyspecific immunoconjugate targeted to multidrug
          resistant Pseudomonas aeruginosa were also prepared


=> s l11 and "TcmA"
L24           7 L11 AND "TCMA"

=> dup remove l24
PROCESSING COMPLETED FOR L24
L25           3 DUP REMOVE L24 (4 DUPLICATES REMOVED)

=> d l25 1-3 cbib abs

L25  ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug resistance in
          cancer and infectious lesions using immunoconjugates.  Goldenberg, David
          M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
          40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
          CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
          IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
          MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
          TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
          TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
          GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
          CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
          1998-PV99304 19980904.
AB        Immunoconjugates of a diagnostic agent and an antibody component that
          binds an epitope of a multidrug transporter protein are
          disclosed.  Thesse immunoconjugates are used in in vivo diagnostic methods
          to determine whether the failure of traditional chemotherapy is due to the
          presence of multidrug resistant tumor cells, multidrug resistant
          HIV-infected cells or multidrug resistant infectious agents.

L25  ANSWER 2 OF 3     MEDLINE on STN                    DUPLICATE 1
97160981.    PubMed ID: 9008277.   Mycobacterium tuberculosis efpA encodes an
          efflux protein of the QacA transporter family. Doran J L; Pang
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     Y; Mdluli K E; Moran A J; Victor T C; Stokes R W; Mahenthiralingam E;
     Kreiswirth B N; Butt J L; Baron G S; Treit J D; Kerr V J; Van Helden P D;
     Roberts M C; Nano F E. (Department of Biochemistry and Microbiology,
     University of Victoria, British Columbia, Canada.. jdoran@uvic.ca) .
     Clinical and diagnostic laboratory immunology, (1997 Jan) 4 (1) 23-32.
     Journal code: 9421292. ISSN: 1071-412X. Pub. country: United States.
     Language: English.
AB   The Mycobacterium tuberculosis H37Rv efpA gene encodes a putative efflux
     protein, EfpA, of 55,670 Da.  The deduced EfpA protein was similar in
     secondary structure to Pur8, MmrA, TcmA, LfrA, EmrB, and other
     members of the QacA transporter family (QacA TF) which mediate
     antibiotic and chemical resistance in bacteria and yeast.  The predicted
     EfpA sequence possessed all transporter motifs characteristic of
     the QacA TF, including those associated with proton-antiport function and
     the motif considered to be specific to exporters.  The 1,590-bp efpA open
     reading frame was G+C rich (65%), whereas the 40-bp region immediately
     upstream had an A+T bias (35% G+C).  Reverse transcriptase-PCR assays
     indicated that efpA was expressed in vitro and in situ.  Putative promoter
     sequences were partially overlapped by the A+T-rich region and by a region
     capable of forming alternative secondary structures indicative of
     transcriptional regulation in analogous systems.  PCR single-stranded
     conformational polymorphism analysis demonstrated that these upstream
     flanking sequences and the 231-bp, 5' coding region are highly conserved
     among both drug-sensitive and multiply-drug-resistant isolates of M.
     tuberculosis.  The efpA gene was present in the slow-growing human
     pathogens M. tuberculosis, Mycobacterium leprae, and Mycobacterium bovis
     and in the opportunistic human pathogens Mycobacterium avium and
     Mycobacterium intracellular.  However, efpA was not present in 17 other
     opportunistically pathogenic or nonpathogenic mycobacterial species.

L25  ANSWER 3 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared


=> s l11 and "NorA"
L26        120 L11 AND "NORA"

=> dup remove l26
PROCESSING COMPLETED FOR L26
L27           66 DUP REMOVE L26 (54 DUPLICATES REMOVED)

=> s l27 and antibod?
L28            2 L27 AND ANTIBOD?
```

```
=> dup remove l28
PROCESSING COMPLETED FOR L28
L29            2 DUP REMOVE L28 (0 DUPLICATES REMOVED)

=> d l29 1-2 cbib abs

L29  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
AB   Immunoconjugates of a diagnostic agent and an antibody component
     that binds an epitope of a multidrug transporter protein are
     disclosed.  Thesse immunoconjugates are used in in vivo diagnostic methods
     to determine whether the failure of traditional chemotherapy is due to the
     presence of multidrug resistant tumor cells, multidrug resistant
     HIV-infected cells or multidrug resistant infectious agents.

L29  ANSWER 2 OF 2  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant
     phenotype.  Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int.
     Appl. WO 9604313 A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU,
     BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP,
     KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL,
     PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG,
     CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL,
     PT, SE, SN, TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO
     1995-US9491 19950801.  PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind
     a multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.
     99mTc- and 111Indium-labeled polyspecific immunoconjugate targeted to
     multidrug resistant Pseudomonas aeruginosa were also prepared


=> s l11 and "Cm1A"
L30           0 L11 AND "CM1A"

=> s l11 and "Bcr"
L31          97 L11 AND "BCR"

=> dup remove l31
PROCESSING COMPLETED FOR L31
L32           59 DUP REMOVE L31 (38 DUPLICATES REMOVED)

=> d l32 1-59 cbib

L32  ANSWER 1 OF 59  CAPLUS  COPYRIGHT 2005 ACS on STN
2005:14242  Gene expression profile useful for diagnosis and treatment methods
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related to aging of liver. Kopchick, John J.; Kelder, Bruce; Boyce, Keith S.; Kriete, Andres (Ohio University, USA). PCT Int. Appl. WO 2005000335 A2 20050106, 459 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US17322 20040602. PRIORITY: US 2003-PV474606 20030602.

L32 ANSWER 2 OF 59 CAPLUS COPYRIGHT 2005 ACS on STN
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          KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
          MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN,
          TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ,
          MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES,
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     UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF,
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L33         21 L11 AND "EMRB"

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L34           9 DUP REMOVE L33 (12 DUPLICATES REMOVED)

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United States. Language: English.

AB Antibiotic efflux is an important mechanism of resistance in pathogenic bacteria. Here we describe the identification and characterization of a novel chromosomally encoded multidrug resistance efflux protein in Staphylococcus aureus, MdeA (multidrug efflux A). MdeA was identified from screening an S. aureus open reading frame expression library for resistance to antibiotic compounds. When overexpressed, MdeA confers resistance on S. aureus to a range of quaternary ammonium compounds and antibiotics, but not fluoroquinolones. MdeA is a 52-kDa protein with 14 predicted transmembrane segments. It belongs to the major facilitator superfamily and is most closely related, among known efflux proteins, to LmrB of Bacillus subtilis and **EmrB** of Escherichia coli. Overexpression of mdeA in S. aureus reduced ethidium bromide uptake and enhanced its efflux, which could be inhibited by reserpine and abolished by an uncoupler. The mdeA promoter was identified by primer extension. Spontaneous mutants selected for increased resistance to an MdeA substrate had undergone mutations in the promoter for mdeA, and their mdeA transcription levels were increased by as much as 15-fold. The mdeA gene was present in the genomes of all six strains of S. aureus examined. Uncharacterized homologs of MdeA were present elsewhere in the S. aureus genome, but their overexpression did not mediate resistance to the antibacterials tested. However, MdeA homologs were identified in other bacteria, including Bacillus anthracis, some of which were shown to be functional orthologs of MdeA.

L34 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 2

2003193214. PubMed ID: 12482849. Identification of oligomerization and drug-binding domains of the membrane fusion protein EmrA. Borges-Walmsley M Ines; Beauchamp Jeremy; Kelly Sharon M; Jumel Kornelia; Candlish Denise; Harding Stephen E; Price Nicholas C; Walmsley Adrian R. (Centre for Infectious Diseases, Wolfson Research Institute, University of Durham, Queen's Campus, Stockton-on-Tees, TS17 6BH, United Kingdom.) Journal of biological chemistry, (2003 Apr 11) 278 (15) 12903-12. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Many pathogenic Gram-negative bacteria possess tripartite **transporters** that catalyze drug extrusion across the inner and outer membranes, thereby conferring resistance. These **transporters** consist of inner (IMP) and outer (OMP) membrane proteins, which are coupled by a periplasmic membrane fusion (MFP) protein. However, it is not know whether the MFP translocates the drug between the membranes, by acting as a channel, or whether it brings the IMP and OMP together, facilitating drug transfer. The MFP EmrA has an elongated periplasmic domain, which binds transported drugs, and is anchored to the inner membrane by a single alpha-helix, which contains a leucine zipper dimerization domain. Consistent with CD and hydrodynamic analyses, the periplasmic domain is predicted to be composed of a beta-sheet subdomain and an alpha-helical coiled-coil. We propose that EmrA forms a trimer in which the coiled-coils radiate across the periplasm, where they could sequester the OMP TolC. The "free" leucine zipper in the EmrA trimer might stabilize the interaction with the IMP **EmrB**, which also possesses leucine zipper motifs in the putative N- and C-terminal helices. The beta-sheet subdomain of EmrA would sit at the membrane surface adjacent to the **EmrB**, from which it receives the transported drug, inducing a conformational change that triggers the interaction with the OMP.

L34 ANSWER 3 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:278283 Document No.: PREV200200278283. Microbial multidrug efflux: Introduction. Paulsen, Ian T. [Reprint author]; Lewis, Kim. The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD, 20850, USA. ipaulsen@tigr.org. Paulsen, Ian T. [Editor]; Lewis, Kim [Editor]. (2002) pp. 1-4. JMMB Symposium Series. Microbial multidrug efflux. print. Publisher: Horizon Scientific Press, 32 Hewitts Lane, Wymondham, Norfolk, NR18 0JA, UK. Series: JMMB Symposium Series. ISBN: 1-898486-33-6 (cloth). Language: English.

L34 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
2002:121138 Document No. 137:104513 A genome-scale analysis for identification of genes required for growth or survival of Haemophilus influenzae. Akerley, Brian J.; Rubin, Eric J.; Novick, Veronica L.; Amaya, Kensey; Judson, Nicholas; Mekalanos, John J. (Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, 48109, USA). Proceedings of the National Academy of Sciences of the United States of America, 99(2), 966-971 (English) 2002. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB A high-d. transposon mutagenesis strategy was applied to the Haemophilus influenzae genome to identify genes required for growth or viability. This anal. detected putative essential roles for the products of 259 ORFs of unknown function. Comparisons between complete genomes defined a subset of these proteins in H. influenzae having homologs in Mycobacterium tuberculosis that are absent in Saccharomyces cerevisiae, a distribution pattern that favors their use in development of antimicrobial therapeutics. Three genes within this set are essential for viability in other bacteria. Interfacing the set of essential gene products in H. influenzae with the distribution of homologs in other microorganisms can detect components of unrecognized cellular pathways essential in diverse bacteria. This genome-scale phenotypic anal. identifies potential roles for a large set of genes of unknown function.

L34 ANSWER 5 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2003:265345 Document No.: PREV200300265345. Identification of a novel multidrug resistance efflux system (MdeA) from Staphylococcus aureus. Huang, J. [Reprint Author]; O'Toole, P. W. [Reprint Author]; Shen, W. [Reprint Author]; Jiang, X. [Reprint Author]; Lobo, N. [Reprint Author]; Palmer, L. M. [Reprint Author]; Voelker, L. [Reprint Author]; Fan, F. [Reprint Author]; Gwynn, M. [Reprint Author]; McDevitt, D. [Reprint Author]. GlaxoSmithKline, Collegeville, PA, USA. Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2002) Vol. 42, pp. 65. print.
Meeting Info.: 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy. San Diego, CA, USA. September 27-30, 2002. American Society for Microbiology.
Language: English.

AB The presence of multidrug efflux pumps in pathogenic bacteria is a major cause of multidrug resistance. Here we report the identification of MdeA, a novel S. aureus efflux system, from screening a S. aureus ORF overexpression library. When over-expressed in S. aureus, MdeA conferred resistance to the antibiotics virginiamycin, novobiocin, and fusidic acid, as well as to the amphipathic cationic compounds ethidium bromide and benzalkonium chloride. Overexpression of MdeA in S. aureus reduced ethidium bromide uptake and accelerated its efflux, which could be inhibited by reserpine and abolished by an uncoupler. Unlike NorA, a known S. aureus efflux pump, MdeA did not confer resistance to fluoroquinolones. Sequence analysis showed that MdeA is composed of 479 amino acid residues with 14 putative transmembrane segments, and it is most closely related, among known efflux proteins, to the **EmrB** protein of Escherichia coli. However, closer homologs of unknown function are present in a range of other bacterial pathogens such as Enterococcus faecalis and Bacillus anthracis. The mdeA gene is present in all publicly available S. aureus genomes. The mdeA promoter was identified by primer extension, and the gene was shown to be weakly transcribed in S. aureus RN4220. However, spontaneous mutants selected for increased resistance to MdeA substrates had undergone mutations in the mdeA promoter region, and mdeA transcription levels in these mutants were increased 6 to 8-fold. Collectively, our data indicate that the novel efflux protein MdeA in S. aureus has the potential to contribute to the drug-resistance phenotype of this and other pathogens.

L34 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in

cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L34 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN

1997:478870 Document No. 127:172150 Cloning and characterization of the bmr3, a third multidrug efflux **transporter** gene of Bacillus subtilis. Murata, Makiko; Ohki, Reiko (Sch. Health Sci., Kyorin Univ., Mitaka, 181, Japan). Kyorin Igakkai Zasshi, 28(2), 155-165 (Japanese) 1997. CODEN: KIZSB8. ISSN: 0368-5829. Publisher: Kyorin Igakkai.

AB A new multidrug **transporter** gene, named bmr3 was cloned from B. subtilis. The bmr3 gene encodes 512 amino acids long peptide which is predicted to be an integral membrane protein with 14 transmembrane segments. It shows moderate homol. to **transporter** protein **EmrB** of Escherichia coli and to a lesser extent to **transporter** protein Bmr and Blt of B. subtilis. Bmr protein has 2 consensus sequences which are conserved in several membrane translocases and multidrug **transporters.** B. subtilis transformed with a multicopy plasmid expressing the bmr3 gene displayed a high level of resistance to tosufloxacin and puromycin and low but significant resistance to norfloxacin, acriflavine, ethidium bromide, and tetraphenylphosphonium bromide. The substrate specificity of Bmr3 is over lapping with that of Bmr and Blt. Disruption of the bmr3 gene on the chromosome did not affect the intrinsic resistance to drugs. The Bmr3 protein was identified by a maxicell method.

L34 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 3

97160981. PubMed ID: 9008277. Mycobacterium tuberculosis efpA encodes an efflux protein of the QacA **transporter** family. Doran J L; Pang Y; Mdluli K E; Moran A J; Victor T C; Stokes R W; Mahenthiralingam E; Kreiswirth B N; Butt J L; Baron G S; Treit J D; Kerr V J; Van Helden P D; Roberts M C; Nano F E. (Department of Biochemistry and Microbiology, University of Victoria, British Columbia, Canada.. jdoran@uvic.ca) . Clinical and diagnostic laboratory immunology, (1997 Jan) 4 (1) 23-32. Journal code: 9421292. ISSN: 1071-412X. Pub. country: United States. Language: English.

AB The Mycobacterium tuberculosis H37Rv efpA gene encodes a putative efflux protein, EfpA, of 55,670 Da. The deduced EfpA protein was similar in secondary structure to Pur8, MmrA, TcmA, LfrA, **EmrB**, and other members of the QacA **transporter** family (QacA TF) which mediate antibiotic and chemical resistance in bacteria and yeast. The predicted EfpA sequence possessed all **transporter** motifs characteristic of the QacA TF, including those associated with proton-antiport function and the motif considered to be specific to exporters. The 1,590-bp efpA open reading frame was G+C rich (65%), whereas the 40-bp region immediately upstream had an A+T bias (35% G+C). Reverse transcriptase-PCR assays indicated that efpA was expressed in vitro and in situ. Putative promoter sequences were partially overlapped by the A+T-rich region and by a region capable of forming alternative secondary structures indicative of transcriptional regulation in analogous systems. PCR single-stranded conformational polymorphism analysis demonstrated that these upstream

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     flanking sequences and the 231-bp, 5' coding region are highly conserved
     among both drug-sensitive and multiply-drug-resistant isolates of M.
     tuberculosis.  The efpA gene was present in the slow-growing human
     pathogens M. tuberculosis, Mycobacterium leprae, and Mycobacterium bovis
     and in the opportunistic human pathogens Mycobacterium avium and
     Mycobacterium intracellular.  However, efpA was not present in 17 other
     opportunistically pathogenic or nonpathogenic mycobacterial species.

L34  ANSWER 9 OF 9  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658  Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared


=> s l11 and "EmrD"
L35           8 L11 AND "EMRD"

=> dup remove l35
PROCESSING COMPLETED FOR L35
L36           4 DUP REMOVE L35 (4 DUPLICATES REMOVED)

=> d l36 1-4 cbib abs

L36  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2005 ACS on STN
2003:633922  Document No. 139:174810  Identifying environmentally derived DNA
     molecules or proteins involved in producing natural products.  Macneil,
     Ian; Tiong, Choi Lai Yip; Brown, Kara (Aventis Pharmaceuticals Inc., USA).
       PCT Int. Appl. WO 2003066853 A1 20030814, 45 pp.  DESIGNATED STATES: W:
     AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR,
     CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
     MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG,
     SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW;
     RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB,
     GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 2003-US3902 20030207.  PRIORITY: US
     2002-PV355083 20020208; GB 2002-13616 20020613.
AB   The present invention provides a novel and useful method for determining
whether
     an exogenous DNA mol. encodes for a natural product having bioactivity, or
     a protein having bioactivity.  Also provided is a method for separating
     environmentally derived DNA mols. that encode a natural product having
     bioactivity or a protein involved in the production of a bioactive mol. from
     environmentally derived DNA mols. that do not, and a heretofore unknown
     promoter.
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L36 ANSWER 2 OF 4 MEDLINE on STN DUPLICATE 1

2001532913. PubMed ID: 11566977. Analysis of a complete library of putative drug **transporter** genes in Escherichia coli. Nishino K; Yamaguchi A. (Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567-0047, Japan.) Journal of bacteriology, (2001 Oct) 183 (20) 5803-12. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB The complete sequencing of bacterial genomes has revealed a large number of drug **transporter** genes. In Escherichia coli, there are 37 open reading frames (ORFs) assumed to be drug **transporter** genes on the basis of sequence similarities, although the transport capabilities of most of them have not been established yet. We cloned all 37 putative drug **transporter** genes in E. coli and investigated their drug resistance phenotypes using an E. coli drug-sensitive mutant as a host. E. coli cells transformed with a plasmid carrying one of 20 ORFs, i.e., fsr, mdfA, yceE, yceL, bcr, emrKY, emrAB, **emrD**, yidY, yjiO, ydhE, acrAB, cusA (formerly ybdE), yegMNO, acrD, acrEF, yhiUV, emrE, ydgFE, and ybjYZ, exhibited increased resistance to some of the 26 representative antimicrobial agents and chemical compounds tested in this study. Of these 20 ORFs, cusA, yegMNO, ydgFE, yceE, yceL, yidY, and ybjYZ are novel drug resistance genes. The fsr, bcr, yjiO, ydhE, acrD, and yhiUV genes gave broader resistance spectra than previously reported.

L36 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN

2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L36 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN

1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US9491 19950801. PRIORITY: US 1994-286430 19940805.

AB Polyspecific immunoconjugates and antibody composites that bind a multidrug **transporter** protein and an antigen associated with a tumor or infectious agent are used to overcom the multidrug resistant phenotype. These immunoconjugates and composites also can be used diagnostically to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents. In example, polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen bispecific antibody were prepared for colon cancer treatment. 99mTc- and 111Indium-labeled polyspecific immunoconjugate targeted to multidrug

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     resistant Pseudomonas aeruginosa were also prepared


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L37          25 L11 AND "ACRE"

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L38           25 DUP REMOVE L37 (0 DUPLICATES REMOVED)

=> d l38 1-25 cbib abs

L38  ANSWER 1 OF 25      MEDLINE on STN
2004370808.     PubMed ID: 15155734.    AcrA, AcrB, and TolC of Escherichia coli
     Form a Stable Intermembrane Multidrug Efflux Complex. Tikhonova Elena B;
     Zgurskaya Helen I. (Department of Chemistry and Biochemistry, University
     of Oklahoma, Norman, 73019, USA. ) Journal of biological chemistry, (2004
     Jul 30) 279 (31) 32116-24.  Journal code: 2985121R. ISSN: 0021-9258. Pub.
     country: United States. Language: English.
AB   Many transporters of Gram-negative bacteria involved in the
     extracellular secretion of proteins and the efflux of toxic molecules
     operate by forming intermembrane complexes.  These complexes are proposed
     to span both inner and outer membranes and create a bridge across the
     periplasm.  In this study, we analyzed interactions between the inner and
     outer membrane components of the tri-partite multidrug efflux pump
     AcrAB-TolC from Escherichia coli.  We found that, once assembled, the
     intermembrane AcrAB-TolC complex is stable during the separation of the
     inner and outer membranes and subsequent purification.  All three
     components of the complex co-purify when the affinity tag is attached to
     either of the proteins suggesting bi-partite interactions between AcrA,
     AcrB, and TolC.  We show that antibiotics, the substrates of AcrAB-TolC,
     stabilize interactions within the complex.  However, the formation of the
     AcrAB-TolC complex does not require an input of energy.

L38  ANSWER 2 OF 25      MEDLINE on STN
2004017389.     PubMed ID: 14714867.    The phytoalexin-inducible multidrug
     efflux pump AcrAB contributes to virulence in the fire blight pathogen,
     Erwinia amylovora. Burse Antje; Weingart Helge; Ullrich Matthias S.
     (School of Engineering and Sciences, International University Bremen,
     Campusring 1, 28759 Bremen, Germany. ) Molecular plant-microbe
     interactions : MPMI, (2004 Jan) 17 (1) 43-54.  Journal code: 9107902.
     ISSN: 0894-0282. Pub. country: United States. Language: English.
AB   The enterobacterium Erwinia amylovora causes fire blight on members of the
     family Rosaceae, with economic importance on apple and pear.  During
     pathogenesis, the bacterium is exposed to a variety of plant-borne
     antimicrobial compounds.  In plants of Rosaceae, many constitutively
     synthesized isoflavonoids affecting microorganisms were identified.
     Bacterial multidrug efflux transporters which mediate resistance
     toward structurally unrelated compounds might confer tolerance to these
     phytoalexins.  To prove this hypothesis, we cloned the acrAB locus from E.
     amylovora encoding a resistance nodulation division-type transport system.
     In Escherichia coli, AcrAB of E. amylovora conferred resistance to
     hydrophobic and amphiphilic toxins.  An acrB-deficient E. amylovora mutant
     was impaired in virulence on apple rootstock MM 106.  Furthermore, it was
     susceptible toward extracts of leaves of MM 106 as well as to the apple
     phytoalexins phloretin, naringenin, quercetin, and (+)-catechin.  The
     expression of acrAB was determined using the promoterless reporter gene
     egfp.  The acrAB operon was up-regulated in vitro by the addition of
     phloretin and naringenin.  The promoter activity of acrR, encoding a
     regulatory protein involved in acrAB expression, was increased by
     naringenin.  In planta, an induction of acrAB was proved by confocal laser
     scanning microscopy.  Our results strongly suggest that the AcrAB
     transport system plays an important role as a protein complex required for
     virulence of E. amylovora in resistance toward apple phytoalexins and that
     it is required for successful colonization of a host plant.
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L38 ANSWER 3 OF 25 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:782831 The Genuine Article (R) Number: 719AR. Chimeric analysis of AcrA function reveals the importance of its c-terminal domain in its interaction with the AcrB multidrug efflux pump. Elkins C A; Nikaido H (Reprint). Univ Calif Berkeley, Dept Mol & Cell Biol, 426 Barker Hall, Berkeley, CA 94720 USA (Reprint); Univ Calif Berkeley, Dept Mol & Cell Biol, Berkeley, CA 94720 USA. JOURNAL OF BACTERIOLOGY (SEP 2003) Vol. 185, No. 18, pp. 5349-5356. Publisher: AMER SOC MICROBIOLOGY. 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. ISSN: 0021-9193. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB AcrAB-TolC is the major, constitutively expressed efflux protein complex that provides resistance to a variety of antimicrobial agents in Escherichia coli. Previous studies showed that AcrA, a periplasmic protein of the membrane fusion protein family, could function with at least two other resistance-nodulation-division family pumps, AcrD and AcrF, in addition to its cognate partner, AcrB. We found that, among other E. coli resistance-nodulation-division pumps, YhiV, but not MdtB or MdtC, could also function with AcrA. When AcrB was assessed for the capacity to function with AcrA homologs, only **AcrE**, but not YbiU or MdtA, could complement an AcrA deficiency. Since AcrA could, but YhiU could not, function with AcrB, we engineered a series of chimeric mutants of these proteins in order to determine the domain(s) of AcrA that is required for its support of AcrB function. The 290-residue N-terminal segment of the 398-residue protein AcrA could be replaced with a sequence coding for the corresponding region of YhiU, but replacement of the region between residues 290 and 357 produced a protein incapable of functioning with AcrB. In contrast, the replacement of residues 357 through 397 of AcrA still produced a functional protein. We conclude that a small region of AcrA close to, but not at, its C terminus is involved in the interaction with its cognate pump protein, AcrB.

L38 ANSWER 4 OF 25 MEDLINE on STN

2003218128. PubMed ID: 12738864. Structural basis of multiple drug-binding capacity of the AcrB multidrug efflux pump. Yu Edward W; McDermott Gerry; Zgurskaya Helen I; Nikaido Hiroshi; Koshland Daniel E Jr. (Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3202, USA.) Science, (2003 May 9) 300 (5621) 976-80. Journal code: 0404511. ISSN: 1095-9203. Pub. country: United States. Language: English.

AB Multidrug efflux pumps cause serious problems in cancer chemotherapy and treatment of bacterial infections. Yet high-resolution structures of ligand **transporter** complexes have previously been unavailable. We obtained x-ray crystallographic structures of the trimeric AcrB pump from Escherichia coli with four structurally diverse ligands. The structures show that three molecules of ligands bind simultaneously to the extremely large central cavity of 5000 cubic angstroms, primarily by hydrophobic, aromatic stacking and van der Waals interactions. Each ligand uses a slightly different subset of AcrB residues for binding. The bound ligand molecules often interact with each other, stabilizing the binding.

L38 ANSWER 5 OF 25 MEDLINE on STN

2003409962. PubMed ID: 12948774. Multidrug-exporting secondary **transporters.** Murakami Satoshi; Yamaguchi Akihito. (Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, 567-0047, Osaka, Japan.. mura@sanken.osaka-u.ac.jp) . Current opinion in structural biology, (2003 Aug) 13 (4) 443-52. Ref: 50. Journal code: 9107784. ISSN: 0959-440X. Pub. country: England: United Kingdom. Language: English.

AB The major cause of intrinsic drug resistance in Gram-negative bacteria is a resistance nodulation division type multidrug exporter, which couples with an outer membrane channel and a membrane fusion protein and exports drugs out of the cell, bypassing the periplasm; this process is driven by proton motive force. A recent crystal structure determination of a major resistance nodulation division type multidrug exporter, AcrB in Escherichia coli, greatly advances our understanding of the multidrug export mechanism. The most striking feature of the AcrB trimer is the presence of three vestibules open to the periplasm at the boundary between the periplasmic headpiece and the transmembrane region. Substrates can gain access to the central cavity from the periplasmic surface of the cytoplasmic membrane and are then actively transported through the extramembrane pore into the outer membrane channel TolC, via the funnel at the top of the AcrB headpiece.

L38 ANSWER 6 OF 25 MEDLINE on STN
2003208710. PubMed ID: 12729934. Escherichia coli lacking the AcrAB multidrug efflux pump also lacks nonproteinaceous, PHB-polyphosphate Ca2+ channels in the membrane. Jones Helen E; Holland I Barry; Jacq Annicq; Wall Torun; Campbell Anthony K. (Department of Medical Biochemistry, University of Wales College of Medicine, Cardiff, UK.) Biochimica et biophysica acta, (2003 May 2) 1612 (1) 90-7. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB PHB(polyP) complexes bind calcium and form calcium channels in the cytoplasmic membrane in Escherichia coli and are likely to be important in Ca(2+) homeostasis in this organism. E. coli N43, which lacks the AcrA component of a major multidrug resistance pump, was shown to be defective in calcium handling, with an inability to maintain submicromolar levels of free Ca(2+) in the cytoplasm. Therefore, using an N-phenyl-1-napthylamine (NPN)-dependent fluorescence assay, we measured temperature-dependent phase transitions in the membranes of intact cells. These transitions specifically depend on the presence of PHB(Ca(2+)polyP) complexes. PHB(Ca(2+)polyP) channel complexes, particularly in stationary phase cultures, were detected in wild-type strains; however, in contrast, isogenic acrA(-) strains had greatly reduced amounts of the complexes. This indicates that the AcrAB **transporter** may have a novel, hitherto undetected physiological role, either directly in the membrane assembly of the PHB complexes or the transport of a component of the membrane, which is essential for assembly of the complexes into the membrane. In other experiments, we showed that the particular defective calcium handling detected in N43 was not due to the absence of AcrA but to other unknown factors in this strain.

L38 ANSWER 7 OF 25 MEDLINE on STN
2003094192. PubMed ID: 12607261. Crystal structure of bacterial multidrug efflux **transporter** AcrB. Murakami Satoshi; Yamaguchi Akihito. Tanpakushitsu kakusan koso. Protein, nucleic acid, enzyme, (2003 Jan) 48 (1) 26-32. Ref: 26. Journal code: 0413762. ISSN: 0039-9450. Pub. country: Japan. Language: Japanese.

L38 ANSWER 8 OF 25 MEDLINE on STN
2003021415. PubMed ID: 12526851. The structure and function of drug pumps: an update. McKeegan Kenneth S; Borges-Walmsley M Ines; Walmsley Adrian R. (Centre for Infectious Diseases, Wolfson Research Institute, Queen's Campus, University of Durham, Stockton-on-Tees, UK.) Trends in microbiology, (2003 Jan) 11 (1) 21-9. Ref: 56. Journal code: 9310916. ISSN: 0966-842X. Pub. country: England: United Kingdom. Language: English.

AB Our understanding of the exact mechanisms used by the transmembrane protein pumps that confer cellular resistance to cytotoxic drugs has improved enormously with the recent determination of the structures of three Escherichia coli **transporters**, two belonging to the ATP-binding cassette (ABC) superfamily and one to the resistance-nodulation-cell division (RND) family. Although these studies do not provide an insight into how drug pumps can recognize several structurally unrelated drugs, important advances have been also made in this area. Information on the molecular basis of multidrug recognition has been provided by determining the structure of transcriptional regulators that can bind, often structurally unrelated, cytotoxic drugs and control the expression of drug pumps.

L38 ANSWER 9 OF 25 MEDLINE on STN

2003139504. PubMed ID: 12654283. 3D structure of AcrB: the archetypal multidrug efflux **transporter** of Escherichia coli likely captures substrates from periplasm. Elkins Christopher A; Nikaido Hiroshi. (Department of Molecular and Cell Biology, 426, Barker Hall, University of California, Berkeley, CA 94720-3202, USA.) Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy, (2003 Feb) 6 (1) 9-13. Ref: 17. Journal code: 9815369. ISSN: 1368-7646. Pub. country: Scotland: United Kingdom. Language: English.

AB Recent advances in structural biology have extended our understanding of the multiple drug efflux complex, AcrAB-TolC, of Escherichia coli. This tripartite complex and its homologs are the major mechanisms that give most Gram-negative bacteria their characteristic intrinsic resistance to a variety of lipophilic drugs, dyes, and detergents. Most recently, the structure of the **transporter** AcrB was elucidated at high resolution [Nature 419(2002)587]. It is a particularly significant achievement since integral membrane proteins are notoriously elusive structures for crystallography. The striking features of this trimeric pump, such as the presence of potential substrate-binding sites in the periplasmic domain and the possibility of direct interaction with the end of TolC tunnel, refine our understanding of the mode of action of this tripartite efflux transport complex.

L38 ANSWER 10 OF 25 MEDLINE on STN

2002666085. PubMed ID: 12426337. Chimeric analysis of the multicomponent multidrug efflux **transporters** from gram-negative bacteria. Tikhonova Elena B; Wang Quiju; Zgurskaya Helen I. (Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, USA.) Journal of bacteriology, (2002 Dec) 184 (23) 6499-507. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB Many multidrug **transporters** from gram-negative bacteria belong to the resistance-nodulation-cell division (RND) superfamily of **transporters.** RND-type multidrug **transporters** have an extremely broad substrate specificity and protect bacterial cells from the actions of antibiotics on both sides of the cytoplasmic membrane. They usually function as three-component assemblies spanning the outer and cytoplasmic membranes and the periplasmic space of gram-negative bacteria. The structural determinants of RND **transporters** responsible for multidrug recognition and complex assembly remain unknown. We constructed chimeric RND **transporters** composed of N-terminal residues of AcrB and C-terminal residues of MexB, the major RND-type **transporters** from Escherichia coli and Pseudomonas aeruginosa, respectively. The assembly of complexes and multidrug efflux activities of chimeric **transporters** were determined by coexpression of hybrid genes either with AcrA, the periplasmic component of the AcrAB **transporter** from E. coli, or with MexA and OprM, the accessory proteins of the MexAB-OprM pump from P. aeruginosa. We found that the specificity of interaction with the corresponding periplasmic component is encoded in the T60-V612 region of **transporters.** Our results also suggest that the large periplasmic loops of RND-type **transporters** are involved in multidrug recognition and efflux.

L38 ANSWER 11 OF 25 MEDLINE on STN

2002666084. PubMed ID: 12426336. Substrate specificity of the RND-type multidrug efflux pumps AcrB and AcrD of Escherichia coli is determined predominantly by two large periplasmic loops. Elkins Christopher A; Nikaido Hiroshi. (Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3206, USA.) Journal of bacteriology, (2002 Dec) 184 (23) 6490-8. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB AcrAB-TolC is a constitutively expressed, tripartite efflux **transporter** complex that functions as the primary resistance mechanism to lipophilic drugs, dyes, detergents, and bile acids in Escherichia coli. TolC is an outer membrane channel, and AcrA is an elongated lipoprotein that is hypothesized to span the periplasm and coordinate efflux of such substrates by AcrB and TolC. AcrD is an efflux **transporter** of E. coli that provides resistance to aminoglycosides as well as to a limited range of amphiphilic agents, such as bile acids, novobiocin, and fusidic acid. AcrB and AcrD belong to the resistance nodulation division superfamily and share a similar topology, which includes a pair of large periplasmic loops containing more than 300 amino acid residues each. We used this knowledge to test several plasmid-encoded chimeric constructs of acrD and acrB for substrate specificity in a marR1 DeltaacrB DeltaacrD host. AcrD chimeras were constructed in which the large, periplasmic loops between transmembrane domains 1 and 2 and 7 and 8 were replaced with the corresponding loops of AcrB. Such constructs provided resistance to AcrB substrates at levels similar to native AcrB. Conversely, AcrB chimeras containing both loops of AcrD conferred resistance only to the typical substrates of AcrD. These results cannot be explained by simply assuming that AcrD, not hitherto known to interact with AcrA, acquired this ability by the introduction of the loop regions of AcrB, because (i) both AcrD and AcrA were found, in this study, to be required for the efflux of amphiphilic substrates, and (ii) chemical cross-linking in intact cells efficiently produced complexes between AcrD and AcrA. Since AcrD can already interact with AcrA, the alterations in substrate range accompanying the exchange of loop regions can only mean that substrate recognition (and presumably binding) is determined largely by the two periplasmic loops.

L38 ANSWER 12 OF 25 MEDLINE on STN
2002490420. PubMed ID: 12351840. Purification, crystallization and preliminary diffraction studies of AcrB, an inner-membrane multi-drug efflux protein. Pos Klaas M; Diederichs Kay. (Institut fur Mikrobiologie, D-Biol, ETH Zurich, Schmelzbergstrasse 7, CH-8092 Zurich, Switzerland.. pos@micro.biol.ethz.ch) . Acta crystallographica. Section D, Biological crystallography, (2002 Oct) 58 (Pt 10 Pt 2) 1865-7. Journal code: 9305878. ISSN: 0907-4449. Pub. country: Denmark. Language: English.

AB Resistance of pathogens to antibiotics is often dependent on multi-drug export proteins that reside in the inner membrane of bacteria. This work describes the expression, purification, crystallization and preliminary crystallographic analysis of AcrB of Escherichia coli. Together with AcrA and TolC, AcrB forms a proton motive force dependent efflux pump of the resistance-nodulation-cell division (RND) **transporter** superfamily and is responsible for resistance towards many common antibiotics such as ciprofloxacin and novobiocin. AcrB crystallizes in space group R32, with unit-cell parameters a = b = 143, c = 513 A; the crystals diffract to 3.0 A resolution.

L38 ANSWER 13 OF 25 MEDLINE on STN
2002631107. PubMed ID: 12374972. Crystal structure of bacterial multidrug efflux **transporter** AcrB. Murakami Satoshi; Nakashima Ryosuke; Yamashita Eiki; Yamaguchi Akihito. (Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567-0047, Japan.) Nature, (2002 Oct 10) 419 (6907) 587-93. Journal code: 0410462. ISSN: 0028-0836. Pub. country: England: United Kingdom. Language: English.

AB AcrB is a major multidrug exporter in Escherichia coli. It cooperates with a membrane fusion protein, AcrA, and an outer membrane channel, TolC. We have determined the crystal structure of AcrB at 3.5 A resolution. Three AcrB protomers are organized as a homotrimer in the shape of a jellyfish. Each protomer is composed of a transmembrane region 50 A thick and a 70 A protruding headpiece. The top of the headpiece opens like a funnel, where TolC might directly dock into AcrB. A pore formed by three alpha-helices connects the funnel with a central cavity located at the bottom of the headpiece. The cavity has three vestibules at the side of the headpiece which lead into the periplasm. In the transmembrane region, each protomer has twelve transmembrane alpha-helices. The structure implies that substrates translocated from the cell interior through the transmembrane region and from the periplasm through the vestibules are collected in the central cavity and then actively transported through the pore into the TolC tunnel.

L38 ANSWER 14 OF 25 MEDLINE on STN
2002045516. PubMed ID: 11754746. Membrane topology of a multidrug efflux **transporter**, AcrB, in Escherichia coli. Fujihira Erika; Tamura Norihisa; Yamaguchi Akihito. (Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567-0047, Japan.) Journal of biochemistry, (2002 Jan) 131 (1) 145-51. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB AcrA/B in Escherichia coli is a multicomponent system responsible for intrinsic resistance to a wide range of toxic compounds, and probably cooperates with the outer membrane protein TolC. In this study, acrAB genes were cloned from the E. coli W3104 chromosome. To determine the topology of the inner membrane component AcrB, we employed a chemical labeling approach to analyse mutants of AcrB in which a single cysteine residue had been introduced. The cysteine-free AcrB mutant, in which the two intrinsic Cys residues were replaced by Ala, retained full drug resistance. We constructed 33 cysteine mutants in which a single cysteine was introduced into each putative hydrophilic loop region of the cysteine-free AcrB. The binding of [(14)C]N-ethylmaleimide (NEM) to the Cys residue and the competition of NEM binding with the binding of a membrane-impermeant maleimide, 4-acetamide-4'-maleimidylstilbene-2,2'-disulfonic acid (AMS), in intact cells were investigated. The results revealed that the N- and C-terminals are localized on the cytoplasmic surface of the membrane and the two large loops are localized on the periplasmic surface of the membrane. The results supported the 12-membrane-spanning structure of AcrB. Three of the four short periplasmic loop regions were covered by the two large periplasmic loop domains and were not exposed to the water phase until one of the two large periplasmic loops was removed.

L38 ANSWER 15 OF 25 MEDLINE on STN
2001701350. PubMed ID: 11741850. Genetic screen yields mutations in genes encoding all known components of the Escherichia coli signal recognition particle pathway. Tian Hongping; Beckwith Jon. (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA.) Journal of bacteriology, (2002 Jan) 184 (1) 111-8. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB We describe the further utilization of a genetic screen that identifies mutations defective in the assembly of proteins into the Escherichia coli cytoplasmic membrane. The screen yielded mutations in each of the known genes encoding components of the E. coli signal recognition particle pathway: ffh, ffs, and ftsY, which encode Ffh, 4.5S RNA, and FtsY, respectively. In addition, the screen yielded mutations in secM, which is involved in regulating levels of the SecA component of the bacterium's protein export pathway. We used a sensitive assay involving biotinylation to show that all of the mutations caused defects in the membrane insertions of three topologically distinct membrane proteins, AcrB, MalF, and FtsQ. Among the mutations that resulted in membrane protein insertion defects, only the secM mutations also showed defects in the translocation of proteins into the E. coli periplasm. Genetic evidence suggests that the S382T alteration of Ffh affects the interaction between Ffh and 4.5S RNA.

L38 ANSWER 16 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
2001:634533 Document No. 136:242629 The complete sequence of the 1,683-Kb pSymB megaplasmid from the N2-fixing endosymbiont Sinorhizobium meliloti. Finan, Turlough M.; Weidner, Stefan; Wong, Kim; Buhrmester, Jens; Chain, Patrick; Vorholter, Frank J.; Hernandez-Lucas, Ismael; Becker, Anke; Cowie, Alison; Gouzy, Jerome; Golding, Brian; Puhler, Alfred (Department of Biology, McMaster University, Hamilton, ON, L8S 4K1, Can.). Proceedings of the National Academy of Sciences of the United States of

America, 98(17), 9889-9894 (English) 2001. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB Anal. of the 1683,333-nt sequence of the pSymB megaplasmid from the symbiotic N2-fixing bacterium Sinorhizobium meliloti revealed that the replicon has a high gene d. with a total of 1570 protein-coding regions, with few insertion elements and regions duplicated elsewhere in the genome. The only copies of an essential arg-tRNA gene and the minCDE genes are located on pSymB. Almost 20% of the pSymB sequence carries genes encoding solute uptake systems, most of which were of the ATP-binding cassette family. Many previously unsuspected genes involved in polysaccharide biosynthesis were identified and these, together with the two known distinct exopolysaccharide synthesis gene clusters, show that 14% of the pSymB sequence is dedicated to polysaccharide synthesis. Other recognizable gene clusters include many involved in catabolic activities such as protocatechuate utilization and phosphonate degradation The functions of these genes are consistent with the notion that pSymB plays a major role in the saprophytic competence of the bacteria in the soil environment.

L38 ANSWER 17 OF 25 MEDLINE on STN

2002007217. PubMed ID: 11248102. The targeting pathway of Escherichia coli presecretory and integral membrane proteins is specified by the hydrophobicity of the targeting signal. Lee H C; Bernstein H D. (Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Building 10, Room 9D-20, Bethesda, MD 20892-1810, USA.) Proceedings of the National Academy of Sciences of the United States of America, (2001 Mar 13) 98 (6) 3471-6. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Previous studies have demonstrated that presecretory proteins such as maltose binding protein (MBP) and outer membrane protein A (OmpA) are targeted to the Escherichia coli inner membrane by the molecular chaperone SecB, but that integral membrane proteins are targeted by the signal recognition particle (SRP). In vitro studies have suggested that trigger factor binds to a sequence near the N terminus of the mature region of OmpA and shunts the protein into the SecB pathway by blocking an interaction between SRP and the signal peptide. By contrast, we have found that the targeting pathway of a protein under physiological conditions is dictated by the composition of its targeting signal. Replacement of the MBP or OmpA signal peptide with the first transmembrane segment of AcrB abolished the dependence on SecB for transport and rerouted both proteins into the SRP targeting pathway. More modest alterations of the MBP signal peptide that simply increase its hydrophobicity also promoted SRP binding. Furthermore, we obtained evidence that SRP has a low affinity for typical signal peptides in vivo. These results imply that different classes of E. coli proteins are targeted by distinct pathways because bacterial SRP binds to a more restricted range of targeting signals than its eukaryotic counterpart.

L38 ANSWER 18 OF 25 MEDLINE on STN

2001483527. PubMed ID: 11321576. AcrAB and related multidrug efflux pumps of Escherichia coli. Nikaido H; Zgurskaya H I. (Department of Molecular and Cell Biology, University of California, Berkeley 94720-3206, USA.. nhiroshi@uclink4.berkeley.edu) . Journal of molecular microbiology and biotechnology, (2001 Apr) 3 (2) 215-8. Ref: 29. Journal code: 100892561. ISSN: 1464-1801. Pub. country: England: United Kingdom. Language: English.

AB The AcrAB system of Escherichia coli is a multidrug efflux system composed of an RND-type **transporter** AcrB and a periplasmic accessory protein AcrA, and pumps out a wide variety of lipophilic and amphiphilic inhibitors directly into the medium, presumably through the TolC outer membrane channel. AcrA, a highly elongated protein, is thought to bring the outer and inner membranes closer. It forms a trimer that interacts with a monomeric AcrB, which was shown by in vitro reconstitution to be a proton antiporter. Details of interaction between the

L38 ANSWER 19 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L38 ANSWER 20 OF 25 MEDLINE on STN
2000396410. PubMed ID: 10894736. Cross-linked complex between oligomeric periplasmic lipoprotein AcrA and the inner-membrane-associated multidrug efflux pump AcrB from Escherichia coli. Zgurskaya H I; Nikaido H. (Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3206, USA.) Journal of bacteriology, (2000 Aug) 182 (15) 4264-7. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB In Escherichia coli, the intrinsic levels of resistance to multiple antimicrobial agents are produced through expression of the three-component multidrug efflux system AcrAB-TolC. AcrB is a proton-motive-force-dependent **transporter** located in the inner membrane, and AcrA and TolC are accessory proteins located in the periplasm and the outer membrane, respectively. In this study, these three proteins were expressed separately, and the interactions between them were analyzed by chemical cross-linking in intact cells. We show that AcrA protein forms oligomers, most probably trimers. In this oligomeric form, AcrA interacts specifically with AcrB **transporter** independently of substrate and TolC.

L38 ANSWER 21 OF 25 CAPLUS COPYRIGHT 2005 ACS on STN
1999:734683 Document No. 132:216310 Resistance to macrolides mediated by efflux mechanisms. Sutcliffe, Joyce (Department of Infectious Diseases, Pfizer Central Research Inc, Groton, CT, 06340, USA). Current Opinion in Anti-Infective Investigational Drugs, 1(4), 403-412 (English) 1999. CODEN: COADFY. ISSN: 1464-8458. Publisher: Current Drugs Ltd..

AB A review with 92 refs. Drug transport proteins can be divided into two classes: secondary **transporters**, which couple extrusion of drugs with ion exchange, and ATP-binding cassette (ABC) **transporters**, which utilize the energy of a phosphate bond upon ATP hydrolysis to efflux drugs. Efflux is mediated by either a pump with narrow specificity, e.g, Mef and MsrA, or a pump with broad specificity, e.g., AcrAB-TolC, AcrEF-TolC, MexAB-OprM, MexCD-OprJ, AmrAB-OprA, MtrCDE, EmrE, MdfA and Cmr. Staphylococcus epidermidis harbors a plasmid with an msrA determinant, an ABC-type **transporter** specific for 14- and 15-membered macrolides and streptogramin B. An almost identical gene resides in Staphylococcus aureus (S aureus), although it appears to be uncommon. MsrA appears to be the ATP binding component of the ABC **transporter** and likely works in concert with various pumps that have been identified on plasmids or on the chromosome of staphylococcal strains. MsrB, found in Staphylococcus xylosus, is identical to the C-terminal portion of MsrA and likely functions as a dimer. MefA and MefE, initially described in Streptococcus pyogenes and Streptococcus pneumoniae, resp., have been folded into a single family, MefA, because the two genes are approx. 90% identical at the amino acid level. The MefA protein is a member of the major facilitator superfamily (MFS) and can efflux 14- and 15- membered macrolides. Resistance to 16-membered macrolides is not seen even in the presence of inducing concns. of erythromycin. Strains harboring the mefA gene remain susceptible to streptogramin B and clindamycin. Interestingly, the MefA protein conferred macrolide resistance when cloned into Escherichia coli (E coli). More recently, mefA has been described in many different streptococcal species, moderately erythromycin-resistant strains of Enterococcus faecium, Micrococcus luteus and Corynebacterium jeikeiium. Other members of the MFS class that can cause the active egress of erythromycin include MdfA in E coli and Cmr in Corynebacterium glutamicum. The latter two require overexpression to confer approx. 4-fold level of resistance. Another putative pump, MreA, was described in a clin. strain of Streptococcus agalactiae, but recent work demonstrates that this protein likely encodes a riboflavin kinase (ribC). The AcrAB-TolC pump is found in E coli and similar members of the resistance-nodulation-cell division family appear to be present in Haemophilus influenzae (AcrAB-like), Pseudomonas aeruginosa (MexAB-OprM, MexCD-OprJ), Burkholderia pseudomallei (AmrAB-OprA) and Neisseria gonorrhoeae (MtrCDE). The **transporters** in the Gram-neg. bacteria (AcrB, AcrF, MexB, MexD, AmrB, MtrD) work in concert with an accessory protein (AcrA, **AcrE**, MexA, MexC, AmrA, MtrC) which spans the periplasmic space and interacts with a porin in the outer membrane (TolC, TolC(), OprM, OprJ, OprA, MtrE). AcrAB-TolC and MtrCDE confer resistance to hydrophobic antibiotics (including erythromycin), detergents and dyes, while the two multidrug pumps from Pseudomonas overlap in their ability to efflux compds. such as tetracycline, novobiocin, chloramphenicol, erythromycin and fluoroquinolones, but show specificity toward certain β-lactams and fourth generation cephalosporins. AmrAB-OprA confers high-level resistance to aminoglycosides and macrolides. Some of these pumps are constitutive, e.g., AcrAB-TolC, AcrAB-Omp2, MexAB-OprM, AmrAB-OprA, while others remain silent until promoter or regulatory mutations allow their expression. Macrolide resistance can also be conferred by members of the small multidrug resistance family, EmrE and Mmr. EmrE is chromosomally encoded in E coli and has no known transmembrane linker or outer membrane channel associated with it. Reconstituted EmrE catalyzes the efflux or erythromycin and tetracycline, in addition to lipophilic monovalent cations. Mmr has been cloned from Mycobacterium tuberculosis and confers erythromycin resistance in Mycobacterium smegmatis. All the resistance determinants are intrinsic and chromosomally encoded, with the exception of msrA and mefA. Recently, mefA was transferred to naive strains by conjugation; it is likely mobile by a chromosomally integrated transposon. MsrA is generally plasmid-mediated. The expression of both genes is inducible by erythromycin.

L38 ANSWER 22 OF 25 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

97:606505 The Genuine Article (R) Number: XP985. Mechanisms of secretion of toxic organic anions in mammalian kidneys. Orlov Y N (Reprint). RUSSIAN ACAD SCI, ST PETERSBURG INST NUCL PHYS, ST PETERSBURG 188350, RUSSIA (Reprint). BIOLOGICHESKIE MEMBRANY (JUL-AUG 1997) Vol. 14, No. 4, pp. 341-350. Publisher: MEZHDUNARODNAYA KNIGA. 39 DIMITROVA UL., 113095 MOSCOW, RUSSIA. ISSN: 0233-4755. Pub. country: RUSSIA. Language: Russian. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB This short review is dedicated to the studies of the mechanisms of organic anion secretion in renal proximal tubules. Particular attention is given to the energetic coupling of p-aminohippurate transport to inorganic anion gradients generated **acres** both basolateral and brush-border membranes, The coupling is considered to be a consequence of the combined operation of the co-transport and anion-exchange mechanisms. A possible coexistence of several organic anion pathways with overlapping substrate specificity and (or) energetic dependence olf the total ion gradient are suggested. The problem of identification of **transporters** involved in the organic anion secretion is discussed.

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L38  ANSWER 23 OF 25  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared

L38  ANSWER 24 OF 25  CAPLUS  COPYRIGHT 2005 ACS on STN
1994:695420   Document No. 121:295420  Czc/Cnr efflux: a three-component
     chemiosmotic antiport pathway with a 12-transmembrane-helix protein.
     Dong, Qinghan; Mergeay, Max (Environmental Technology, VITO, Mol, B-2400,
     Belg.).  Molecular Microbiology, 14(1), 185-7 (English) 1994.  CODEN:
     MOMIEE.  ISSN: 0950-382X.
AB   The authors show that CzcB and CnrB, members of an Alcaligenes eutrophus
     membrane transporter RND family, are also effectively membrane
     fusion proteins just like AcrA, AcrE, or NolF.  Thus, the 3
     components of the Czc/Cnr efflux systems, CzcCBA/CnrCBA, as well as their
     homologs in Pseudomonas aeruginosa and Alcaligenes xylosoxidans belong to
     3 distinct families of membrane proteins.  Partial amino acid sequences of
     6 membrane fusion proteins including CzcB and CnrB are compared.

L38  ANSWER 25 OF 25  CAPLUS  COPYRIGHT 2005 ACS on STN
1994:24616   Document No. 120:24616  Molecular cloning and characterization of
     acrA and acrE genes of Escherichia coli.  Ma, Dzwokai; Cook,
     David N.; Alberti, Marie; Pon, Ning G.; Nikaido, Hiroshi; Hearst, John E.
     (Dep. Chem., Univ. California, Berkeley, CA, 94720, USA).  Journal of
     Bacteriology, 175(19), 6299-313 (English) 1993.  CODEN: JOBAAY.  ISSN:
     0021-9193.
AB   The DNA fragment containing the acrA locus of the Escherichia coli chromosome
     was cloned by using a complementation test.  The nucleotide sequence
     indicates the presence of 2 open reading frames (ORFs).  Sequence anal.
     suggests that the first ORF encodes a 397-residue lipoprotein with a
     24-amino-acid signal peptide at its N terminus.  One inactive allele of
     acrA from strain N43 was shown to contain an IS2 element inserted into
     this ORF.  Therefore, this ORF was designated acrA.  The 2nd downstream
     ORF is predicted to encode a transmembrane protein of 1049 amino acids and
     is named acrE.  Genes acrA and acrE are probably
     located on the same operon, and both of their products are likely to
     affect drug susceptibilities observed in wild-type cells.  The cellular
     localizations of these polypeptides were analyzed by making acrA:TnphoA
     and acrE:TnphoA fusion proteins.  Interestingly, AcrA and
     AcrE share 65 and 77% amino acid identity with 2 other E. coli
     polypeptides, EnvC and EnvD, resp.  Drug susceptibilities in one acrA
     mutant (N43) and one envCD mutant (PM61) were determined and compared.
     Finally, the possible functions of these proteins are discussed.


=> s l11 and "EnvD"
```

```
L39           4 L11 AND "ENVD"

=> dup remove l39
PROCESSING COMPLETED FOR L39
L40           4 DUP REMOVE L39 (0 DUPLICATES REMOVED)

=> d l40 1-4 cbib abs

L40  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017   Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
AB   Immunoconjugates of a diagnostic agent and an antibody component that
     binds an epitope of a multidrug transporter protein are
     disclosed.  Thesse immunoconjugates are used in in vivo diagnostic methods
     to determine whether the failure of traditional chemotherapy is due to the
     presence of multidrug resistant tumor cells, multidrug resistant
     HIV-infected cells or multidrug resistant infectious agents.

L40  ANSWER 2 OF 4  CAPLUS  COPYRIGHT 2005 ACS on STN
1997:585491   Document No. 128:44390  Complete genome sequence of Escherichia
     coli K-12.  Blattner, Frederick R.; Plunkett, Guy, III; Bloch, Craig A.;
     Perna, Nicole T.; Burland, Valerie; Riley, Monica; Collado-Vides, Julio;
     Glasner, Jeremy D.; Rode, Christopher K.; Mayhew, George F.; Gregor,
     Jason; Davis, Nelson Wayne; Kirkpatrick, Heather A.; Goeden, Michael A.;
     Rose, Debra J.; Mau, Bob; Shao, Ying (Lab. Genetics, Univ.
     Wisconsin-Madison, Madison, WI, 53706, USA).  Science (Washington, D. C.),
     277(5331), 1453-1462 (English) 1997.  CODEN: SCIEAS.  ISSN: 0036-8075.
     Publisher: American Association for the Advancement of Science.
AB   The 4,639,221-base pair sequence of Escherichia coli K-12 is presented.
     Of 4288 protein-coding genes annotated, 38 percent have no attributed
     function.  Comparison with five other sequenced microbes reveals
     ubiquitous as well as narrowly distributed gene families; many families of
     similar genes within E. coli are also evident.  The largest family of
     paralogous proteins contains 80 ABC transporters.  The genome as
     a whole is strikingly organized with respect to the local direction of
     replication; guanines, oligonucleotides possibly related to replication
     and recombination, and most genes are so oriented.  The genome also
     contains insertion sequence (IS) elements, phage remnants, and many other
     patches of unusual composition indicating genome plasticity through horizontal
     transfer.

L40  ANSWER 3 OF 4  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared

L40  ANSWER 4 OF 4  CAPLUS  COPYRIGHT 2005 ACS on STN
1994:24616   Document No. 120:24616  Molecular cloning and characterization of
     acrA and acrE genes of Escherichia coli.  Ma, Dzwokai; Cook, David N.;
     Alberti, Marie; Pon, Ning G.; Nikaido, Hiroshi; Hearst, John E. (Dep.
     Chem., Univ. California, Berkeley, CA, 94720, USA).  Journal of
     Bacteriology, 175(19), 6299-313 (English) 1993.  CODEN: JOBAAY.  ISSN:
     0021-9193.
AB   The DNA fragment containing the acrA locus of the Escherichia coli chromosome
     was cloned by using a complementation test.  The nucleotide sequence
     indicates the presence of 2 open reading frames (ORFs).  Sequence anal.
     suggests that the first ORF encodes a 397-residue lipoprotein with a
     24-amino-acid signal peptide at its N terminus.  One inactive allele of
     acrA from strain N43 was shown to contain an IS2 element inserted into
     this ORF.  Therefore, this ORF was designated acrA.  The 2nd downstream
     ORF is predicted to encode a transmembrane protein of 1049 amino acids and
     is named acrE.  Genes acrA and acrE are probably located on the same
     operon, and both of their products are likely to affect drug
     susceptibilities observed in wild-type cells.  The cellular localizations of
     these polypeptides were analyzed by making acrA:TnphoA and acrE:TnphoA
     fusion proteins.  Interestingly, AcrA and AcrE share 65 and 77% amino acid
     identity with 2 other E. coli polypeptides, EnvC and EnvD, resp.
     Drug susceptibilities in one acrA mutant (N43) and one envCD mutant (PM61)
     were determined and compared.  Finally, the possible functions of these
     proteins are discussed.


=> s l11 and "mexB"
L41          75 L11 AND "MEXB"

=> s l11 and "MexB"
L42          75 L11 AND "MEXB"

=> dup remove l42
PROCESSING COMPLETED FOR L42
L43          37 DUP REMOVE L42 (38 DUPLICATES REMOVED)

=> d l43 1-37 cbib abs

L43  ANSWER 1 OF 37  CAPLUS  COPYRIGHT 2005 ACS on STN
2004:271216   Document No. 140:291835  Development of a method for promoting
     microbial biodegradation in bioremediation by using acylated homoserine
     lactones that activate bacterial membrane transport.  Nomura, Nobuhiko;
     Maseda, Hideaki (Nippon Shokubai Co., Ltd., Japan).  Jpn. Kokai Tokkyo
     Koho JP 2004097030 A2 20040402, 14 pp.  (Japanese).  CODEN: JKXXAF.
     APPLICATION: JP 2002-260338 20020905.
GI
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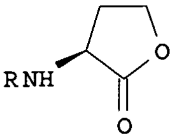

I

AB A method for promoting microbial biodegrdn. in bioremediation by using acylated homoserine lactones I (R = C2 .apprx. C14 linear or branched acyl groups) that activate bacteria has been developed. The method uses recombinant bacteria to which the gene encoding MATE (multidrug and toxic compound extrusion) transport protein has been introduced. Activation of bacteria that results in increase of its bio-decomposing activity is designed to be triggered by the transcriptional activation of the MATE **transporter** gene using I as the inducer. Transcriptional activation of reporter gene inserted in the mexA-**mexB**-oprM operon for MATE **transporter** by N-butyryl-L-homoserine lactone in recombinant Pseudomonas aeruginosa was demonstrated. Promotion of the decomposition of NO2 and ten times-increased denitrification by Burkholderia cepacia to which gene mexEFN for RND family extrusion pump had been introduced was also demonstrated.

L43 ANSWER 2 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2005:11109 The Genuine Article (R) Number: 878XL. Crystal structure of the drug discharge outer membrane protein, OprM, of Pseudomonas aeruginosa - Dual modes of membrane anchoring and occluded cavity end. Akama H; Kanemaki M; Yoshimura M; Tsukihara T; Kashiwagi T; Yoneyama H; Narita S; Nakagawa A (Reprint); Nakae T. Osaka Univ, Inst Prot Res, Suita, Osaka 5650871, Japan (Reprint); Tokai Univ, Sch Med, Dept Mol Life Sci, Isehara, Kanagawa 2591193, Japan. JOURNAL OF BIOLOGICAL CHEMISTRY (17 DEC 2004) Vol. 279, No. 51, pp. 52816-52819. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA. ISSN: 0021-9258. Pub. country: Japan. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The OprM lipoprotein of Pseudomonas aeruginosa is a member of the MexAB-OprM xenobiotic-antibiotic **transporter** subunits that is assumed to serve as the drug discharge duct across the outer membrane. The channel structure must differ from that of the porin-type open pore because the protein facilitates the exit of antibiotics but not the entry. For better understanding of the structure-function linkage of this important pump subunit, we studied the x-ray crystallographic structure of OprM at the 2.56-Angstrom resolution. The overall structure exhibited trimeric assembly of the OprM monomer that consisted mainly of two domains: the membrane-anchoring beta-barrel and the cavity-forming alpha-barrel. OprM anchors the outer membrane by two modes of membrane insertions. One is via the covalently attached NH2-terminal fatty acids and the other is the beta-barrel structure consensus on the outer membrane-spanning proteins. The beta-barrel had a pore opening with a diameter of about 6-8 Angstrom, which is not large enough to accommodate the exit of any antibiotics. The periplasmic alpha-barrel was about 100 Angstrom long formed mainly by a bundle of alpha-helices that formed a solvent-filled cavity of about 25,000 Angstrom(3). The proximal end of the cavity was tightly sealed, thereby not permitting the entry of any molecule. The result of this structure was that the resting state of OprM had a small outer membrane pore and a tightly closed periplasmic end, which sounds plausible because the protein should not allow free access of antibiotics. However, these observations raised another unsolved problem about the mechanism of opening of the OprM cavity ends. The crystal structure offers possible mechanisms of pore opening and pump assembly.

L43 ANSWER 3 OF 37 MEDLINE on STN DUPLICATE 1

2004293190. PubMed ID: 15117957. Crystal structure of the membrane fusion protein, MexA, of the multidrug **transporter** in Pseudomonas aeruginosa. Akama Hiroyuki; Matsuura Takanori; Kashiwagi Sachiko; Yoneyama Hiroshi; Narita Shin-Ichiro; Tsukihara Tomitake; Nakagawa Atsushi; Nakae Taiji. (Department of Molecular Life Science, Tokai University School of Medicine, Isehara, 259-1193, Japan.) Journal of biological chemistry, (2004 Jun 18) 279 (25) 25939-42. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The MexAB-OprM efflux pump of Pseudomonas aeruginosa is central to multidrug resistance of this organism, which infects immunocompromised hospital patients. The MexA, **MexB**, and OprM subunits were assumed to function as the membrane fusion protein, the body of the **transporter**, and the outer membrane channel protein, respectively. For better understanding of this important xenobiotic **transporter** , we show the x-ray crystallographic structure of MexA at a resolution of 2.40 A. The global MexA structure showed unforeseen new features with a spiral assembly of six and seven protomers that were joined together at one end by a pseudo 2-fold image. The protomer showed a new protein structure with a tandem arrangement consisting of at least three domains and presumably one more. The rod domain had a long hairpin of twisted coiled-coil that extended to one end. The second domain adjacent to the rod alpha-helical domain was globular and constructed by a cluster of eight short beta-sheets. The third domain located distal to the alpha-helical rod was globular and composed of seven short beta-sheets and one short alpha-helix. The 13-mer was shaped like a woven rattan cylinder with a large internal tubular space and widely opened flared ends. The 6-mer and 7-mer had a funnel-like structure consisting of a tubular rod at one side and a widely opened flared funnel top at the other side. Based on these results, we constructed a model of the MexAB-OprM pump assembly. The three pairs of MexA dimers interacted with the periplasmic alpha-barrel domain of OprM via the alpha-helical hairpin, the second domain interacted with both **MexB** and OprM at their contact site, and the third and disordered domains probably interacted with the distal domain of **MexB**. In this fashion, the MexA subunit connected **MexB** and OprM, indicating that MexA is the membrane bridge protein.

L43 ANSWER 4 OF 37 MEDLINE on STN DUPLICATE 2
2004083386. PubMed ID: 14973037. Differential impact of **MexB** mutations on substrate selectivity of the MexAB-OprM multidrug efflux pump of Pseudomonas aeruginosa. Middlemiss Jocelyn K; Poole Keith. (Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6.) Journal of bacteriology, (2004 Mar) 186 (5) 1258-69. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB The integral inner membrane resistance-nodulation-division (RND) components of three-component RND-membrane fusion protein-outer membrane factor multidrug efflux systems define the substrate selectivity of these efflux systems. To gain a better understanding of what regions of these proteins are important for substrate recognition, a plasmid-borne **mexB** gene encoding the RND component of the MexAB-OprM multidrug efflux system of Pseudomonas aeruginosa was mutagenized in vitro by using hydroxylamine and mutations compromising the **MexB** contribution to antibiotic resistance identified in a DeltamexB strain. Of 100 mutants that expressed wild-type levels of **MexB** and showed increased susceptibility to one or more of carbenicillin, chloramphenicol, nalidixic acid, and novobiocin, the **mexB** genes of a representative 46 were sequenced, and 19 unique single mutations were identified. While the majority of mutations occurred within the large periplasmic loops between transmembrane segment 1 (TMS-1) and TMS-2 and between TMS-7 and TMS-8 of **MexB**, mutations were seen in the TMSs and in other periplasmic as well as cytoplasmic loops. By threading the **MexB** amino acid sequence through the crystal structure of the homologous RND **transporter** from Escherichia coli, AcrB, a three-dimensional model of a **MexB** trimer was obtained and the mutations were mapped to it. Unexpectedly, most mutations mapped to regions of **MexB** predicted to be involved in trimerization or interaction with MexA rather than to regions expected to contribute to substrate recognition. Intragenic second-site suppressor mutations that restored the activity of the G220S mutant version of **MexB**, which was compromised for resistance to all tested MexAB-OprM antimicrobial substrates, were recovered and mapped to the apparently distal portion of **MexB** that is implicated in OprM interaction. As the G220S mutation likely impacted trimerization, it appears that either proper assembly of the

MexB trimer is necessary for OprM interaction or OprM association with an unstable **MexB** trimer might stabilize it, thereby restoring activity.

L43 ANSWER 5 OF 37 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 3
2005014190 EMBASE [Efflux-mediated antibiotics resistance in bacteria]. POMPES D'EFFLUX ET RESISTANCE AUX ANTIBIOTIQUES CHEZ LES BACTERIES. Cattoir V.. . Vincent.Cattoir@chu-rennes.fr. Pathologie Biologie 52/10 SPEC. ISS. (607-616) 2004.
Refs: 30.
ISSN: 0369-8114. CODEN: PTBIAN.
Publisher Ident.: S 0369-8114(04)00204-4. Pub. Country: France. Language: French. Summary Language: English; French.

AB Bacteria can resist to antibiotics by active exportation mediated by membrane **transporters** called efflux pumps. These proteins can be specific of a class of antibiotics or responsible for multidrug resistance (MDR). Energy required by efflux pumps can be provided by transmembrane electrochemical gradient of protons (MFS, RND, SMR families) or sodium ions (MATE family) or by ATP hydrolysis (ABC family). Several physiological functions have been described in prokaryotes, such as protection from environmental toxics and regulation of cell homeostasis, which can indirectly contributes to bacterial virulence. In Gram-negative bacteria, efflux **transporters** usually are organized as multicomponent systems in wich the efflux pump located in the inner membrane works in conjunction with a periplasmic fusion protein and an outer membrane factor. The most frequently encountered pumps are of the RND-type such as AcrB in Escherichia coli or **MexB** in Pseudomonas aeruginosa. In Gram-positive bacteria, efflux is solely mediated by the pump protein, so described with MFS pumps such as NorA or QacA in Staphylococcus aureus and PmrA in Streptococcus pneumoniae. Efflux **transporters** have also been described in mycobacteria. Although numerous bacterial pumps have been characterized, the clinical consequences of efflux-mediated resistance are mostly unknown because of variable levels of expression and of the lack of specific markers in laboratory practice. Finally, associating pump-specific inhibitors to efflux-sensitive antibiotics might prove an interesting therapeutic perspective. However, inhibitors that are not toxic to eukaryotic cells remain to be identified. .COPYRGT. 2004 Elsevier SAS. Tous droits reserves.

L43 ANSWER 6 OF 37 MEDLINE on STN DUPLICATE 4
2004418509. PubMed ID: 15325256. Role of the membrane fusion protein in the assembly of resistance-nodulation-cell division multidrug efflux pump in Pseudomonas aeruginosa. Mokhonov Vladislav V; Mokhonova Ekaterina I; Akama Hiroyuki; Nakae Taiji. (Department of Molecular Life Science, Tokai University School of Medicine, 143 Shimokasuya, Isehara 259-1193, Japan.) Biochemical and biophysical research communications, (2004 Sep 17) 322 (2) 483-9. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB The tripartite xenobiotic-antibiotic **transporter** of Pseudomonas aeruginosa consists of the inner membrane **transporter** (e.g., **MexB**, MexY), the periplasmic membrane-fusion-protein (e.g., MexA, MexX), and the outer membrane channel protein (e.g., OprM). These subunits were assumed to assemble into a **transporter** unit during export of the substrates. However, subunit interaction and their specificity in native form remained to be elucidated. To address these important questions, we analyzed the role of the individual subunits for the assembly of MexAB-OprM by pull-down assay tagging only one of the subunits. We found stable MexA-**MexB**-OprM complex without chemical cross-linking that withstand all purification procedures. Results of bi-partite interactions analysis showed tight association between MexA and OprM in the absence of **MexB**, whereas the expression systems lacking MexA failed to co-purify **MexB** or OprM. None of the heterologous subunit combinations such as

MexA+MexY(his)+OprM and MexX+**MexB**(his)+OprM showed interaction. These results implied that the membrane fusion protein is central to the tripartite xenobiotic **transporter** assembly.

L43 ANSWER 7 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:558007 The Genuine Article (R) Number: 829UK. Global analysis of the membrane subproteome of Pseudomonas aeruginosa using liquid chromatography-tandem mass. Blonder J; Goshe M B; Xiao W Z; Camp D G; Wingerd M; Davis R W; Smith R D (Reprint). Pacific NW Natl Lab, Environm Mol Sci Lab, POB 999, MSIN K8-98, Richland, WA 99352 USA (Reprint); Pacific NW Natl Lab, Environm Mol Sci Lab, Richland, WA 99352 USA; Pacific NW Natl Lab, Div Biol Sci, Richland, WA 99352 USA. JOURNAL OF PROTEOME RESEARCH (MAY-JUN 2004) Vol. 3, No. 3, pp. 434-444. Publisher: AMER CHEMICAL SOC. 1155 16TH ST, NW, WASHINGTON, DC 20036 USA. ISSN: 1535-3893. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pseudomonas aeruginosa is one of the most significant opportunistic bacterial pathogens in humans causing infections and premature death in patients with cystic fibrosis, AIDS, severe burns, organ transplants, or cancer. Liquid chromatography coupled online with tandem mass spectrometry was used for the large-scale proteomic analysis of the P. aeruginosa membrane subproteome. Concomitantly, an affinity labeling technique, using iodoacetyl-PEO biotin to tag cysteinyl-containing proteins, permitted the enrichment and detection of lower abundance membrane proteins. The application of these approaches resulted in the identification of 786 proteins. A total of 333 proteins (42%) had a minimum of one transmembrane domain (ranging from 1 to 14) and 195 proteins were classified as hydrophobic based on their positive GRAVY values (ranging from 0.01 to 1.32). Key integral inner and outer membrane proteins involved in adaptation and antibiotic resistance were conclusively identified, including the detection of 53% of all predicted opr-type porins (outer integral membrane proteins) and all the components of the mexA-**mexB**-oprM transmembrane protein complex. This work represents one of the most comprehensive proteomic analyses of the membrane subproteome of P. aeruginosa and for prokaryotes in general.

L43 ANSWER 8 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:160338 The Genuine Article (R) Number: 771WQ. Efflux-mediated drug resistance in bacteria. Li X Z; Nikaido H (Reprint). Univ Calif Berkeley, Dept Mol & Cell Biol, 426 Barker Hall, Berkeley, CA 94720 USA (Reprint); Univ Calif Berkeley, Dept Mol & Cell Biol, Berkeley, CA 94720 USA. DRUGS (15 FEB 2004) Vol. 64, No. 2, pp. 159-204. Publisher: ADIS INTERNATIONAL LTD. 41 CENTORIAN DR, PRIVATE BAG 65901, MAIRANGI BAY, AUCKLAND 10, NEW ZEALAND. ISSN: 0012-6667. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Drug resistance in bacteria, and especially resistance to multiple antibacterials, has attracted much attention in recent years. In addition to the well known mechanisms, such as inactivation of drugs and alteration of targets, active efflux is now known to play 9 major role in the resistance of many species to antibacterials. Drug-specific efflux (e.g. that of tetracycline) has been recognised as the major mechanism of resistance to this drug in Gram-negative bacteria. In addition, we now recognise that multidrug efflux pumps are becoming increasingly important. Such pumps play major roles in the antiseptic resistance of Staphylococcus aureus, and fluoroquinolone resistance of S. aureus and Streptococcus pneumoniae. Multidrug pumps, often with very wide substrate specificity, are not only essential for the intrinsic resistance of many Gram-negative bacteria but also produce elevated levels of resistance when overexpressed. Paradoxically, 'advanced' agents for which resistance is unlikely to be caused by traditional mechanisms, such as fluoroquinolones and beta-lactams of the latest generations, are likely to select for overproduction mutants of these pumps and make the bacteria resistant in one step to practically all classes of antibacterial agents. Such overproduction mutants are also selected for by the use of antiseptics and biocides, increasingly incorporated into consumer products, and this is also of major concern. We can consider efflux pumps as potentially effective antibacterial targets. Inhibition of efflux pumps by an efflux pump inhibitor would restore the activity of an agent subject to efflux. An alternative approach is to develop antibacterials that would bypass the action of efflux pumps.

L43 ANSWER 9 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:294280 The Genuine Article (R) Number: 805DJ. TbtABM, a multidrug efflux pump associated with tributyltin resistance in Pseudomonas stutzeri. Jude F; Arpin C (Reprint); Brachet-Castang C; Capdepuy M; Caumette P; Quentin C . Univ Bordeaux 2, Microbiol Lab, 146 Rue Leo Saignat, F-33076 Bordeaux, France (Reprint); Univ Bordeaux 2, Microbiol Lab, F-33076 Bordeaux, France; Univ Bordeaux 1, Lab Oceanog Biol, F-33120 Arcachon, France; Univ Pau, Lab Ecol Mol, F-64000 Pau, France. FEMS MICROBIOLOGY LETTERS (12 MAR 2004) Vol. 232, No. 1, pp. 7-14. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0378-1097. Pub. country: France . Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Tributyltin (TBT) is a toxic agent used in marine antifouling paints. Among the bacterial flora of a polluted harbor, TBT-resistant strains of Pseudomonas stutzeri have been isolated. In the strain 5MP1 (TBT minimum inhibitory concentration (MIC) greater than or equal to 1000 mg 1(-1)), TBT resistance was found to be associated with the presence of the operon tbtABM, homologous to the resistance-nodulation-cell division (RND) efflux pump family, as demonstrated by cloning in Escherichia coli. TbtABM exhibited the greatest homology (60.9-84.9%) with the TtgDEF and SrpABC systems, both involved in aromatic compound tolerance in P. putida. TbtABM conferred multidrug resistance (MDR) including to n-hexane, nalidixic acid, chloramphenicol, and sulfamethoxazole (antibiotic MICs x 4 for the E. coli host strain carrying the operon). By polymerase chain reaction amplification and hybridization experiments, the presence of tbtABM was detected in the TBT-sensitive P. stutzeri 3MP1 (TBT MIC 25 mg 1(-1)). However, the latter strain did not seem to express TbtABM. This is the first description of a MDR efflux pump in P. stutzeri, and of a new kind of substrate, TBT, for the RND family of **transporters.** (C) 2004 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

L43 ANSWER 10 OF 37 MEDLINE on STN DUPLICATE 5

2003269689. PubMed ID: 12795620. Function of the **MexB** efflux-**transporter** divided into two halves. Eda Shima; Yoneyama Hiroshi; Nakae Taiji. (Department of Molecular Life Science, Tokai University School of Medicine, 143 Shimokasuya, Isehara, 259-1193, Japan.) Biochemistry, (2003 Jun 17) 42 (23) 7238-44. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The MexA-**MexB**-OprM efflux pump exports structurally and functionally diverse xenobiotics and confers multi-drug resistance on Pseudomonas aeruginosa cells. The **MexB transporter** traverses the inner membrane twelve times, bears two large periplasmic domains and has two homologous tandem repeats. To test whether two homologous halves of **MexB** function independently or interdependently, the protein was divided medially into two halves, each consisting of six amino- and carboxyl-proximal transmembrane segments. When two halves of **MexB** were coexpressed from independent open reading frames, the cells lacking chromosomal **mexB** exhibited restored antibiotic resistance at a level close to that in the cells producing a full-length **MexB.** In contrast, **MexB** protein containing either an amino- or carboxyl-half fragment failed to transport antibiotics. To test whether the amino- and carboxyl-proximal halves were present in a complex, we purified the histidine-tagged carboxyl-proximal half molecule using nickel-chelate chromatography from the cells that coexpressed two halves. The results showed that the nonhistidine-tagged amino-proximal half was co-purified with the carboxyl-proximal half, thereby indicating that the amino-proximal half fragment was tightly associated with the carboxyl-proximal half molecule. These findings suggest that the presence of both amino- and carboxyl-halves of **MexB** in a complex is essential for transport activity.

L43 ANSWER 11 OF 37 MEDLINE on STN DUPLICATE 6
2003026997. PubMed ID: 12460990. An elegant means of self-protection in gram-negative bacteria by recognizing and extruding xenobiotics from the periplasmic space. Eda Shima; Maseda Hideaki; Nakae Taiji. (Department of Molecular Life Science, Tokai University School of Medicine, 143 Shimokasuya, Isehara 259-1193, Japan.) Journal of biological chemistry, (2003 Jan 24) 278 (4) 2085-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Infection of Pseudomonas aeruginosa in cystic fibrosis patients is a major cause of mortality. This organism shows wide ranging antibiotic resistance that is largely attributable to the expression of xenobiotic efflux pump(s). Here, we show a novel mechanism by which the resistance-nodulation-division-type xenobiotic **transporter** expels potential hazards and protects the interior of the cells. The xenobiotic **transporters MexB** and MexY preferentially export beta-lactam and aminoglycoside antibiotics, respectively. When two large extramembrane loops of MexY were replaced by the corresponding loops of **MexB**, the hybrid protein exhibited beta-lactam selectivity (**MexB**-type), but failed to recognize aminoglycoside. As the transmembrane segment of **MexB** was replaced with a corresponding transmembrane segment of MexY, one-by-one for all 12 segments, all the hybrid proteins showed **MexB**-type antibiotic selectivity. These results clearly demonstrated that the resistance-nodulation-division-type efflux pump in P. aeruginosa selects and transports substrates via the domains that largely protrude over the cytoplasmic membrane. The transmembrane segments were unlikely to have been involved in substrate selectivity. These observations led us to propose a novel mechanism by which the xenobiotic **transporters** in Gram-negative bacteria select and expel substrates from the periplasmic space before potential hazards penetrate into the cytoplasmic membrane.

L43 ANSWER 12 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2003:475927 The Genuine Article (R) Number: 674GM. Antibiotic efflux pumps in prokaryotic cells: occurrence, impact on resistance and strategies for the future of antimicrobial therapy. Van Bambeke F (Reprint); Glupczynski Y; Plesiat P; Pechere J C; Tulkens P M. Univ Catholique Louvain, Unite Pharmacol Cellulaire & Mol, B-1200 Brussels, Belgium (Reprint); Univ Catholique Louvain, Microbiol Lab, Clin Univ Mt Godinne, Yvoir, Belgium; CHU Jean Minjoz, Bacteriol Lab, Besancon, France; Univ Geneva, Dept Microbiol, Geneva, Switzerland. JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY (MAY 2003) Vol. 51, No. 5, pp. 1055-1065. Publisher: OXFORD UNIV PRESS. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND. ISSN: 0305-7453. Pub. country: Belgium; France; Switzerland. Language: English.

L43 ANSWER 13 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2003:800699 The Genuine Article (R) Number: 719VX. Mechanism of antibiotic efflux in gram-negative bacteria. Zgurskaya H I (Reprint); Krishnamoorthy G; Tikhonova E B; Lau S Y; Stratton K L. Univ Oklahoma, Dept Chem & Biochem, 620 Parrington Oval, Rm 208, Norman, OK 73019 USA (Reprint); Univ Oklahoma, Dept Chem & Biochem, Norman, OK 73019 USA. FRONTIERS IN BIOSCIENCE (SEP 2003) Vol. 8, pp. S862-S873. Publisher: FRONTIERS IN BIOSCIENCE INC. C/O NORTH SHORE UNIV HOSPITAL, BIOMEDICAL RESEARCH CENTER, 350 COMMUNITY DR, MANHASSET, NY 11030 USA. ISSN: 1093-9946. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Active efflux of antibiotics mediated by multidrug **transporters** is a mechanistic basis of multidrug resistance in bacteria. The most versatile multidrug **transporters** are those found in Gram-negative bacteria. They have a high level of constitutive expression and provide an immediate response to structurally diverse antimicrobial agents including clinically important antibiotics. The versatility and efficiency of multidrug **transporters** in Gram-negative bacteria heavily depend on coupling of drug efflux with the transport across the outer membrane. The coupling is achieved through the assembly of multi-component protein complexes that span both the inner and the outer membranes of Gram-negative bacteria. In this review we discuss the mechanistic and structural features of multidrug efflux complexes with the major focus on the tight coupling of drug efflux with transport across the outer membrane.

L43 ANSWER 14 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:7309 The Genuine Article (R) Number: 750FU. Mechanism of adaptive resistance to aminoglycosides of Pseudomonas aeruginosa. Hocquet D (Reprint); El Garch F; Vogne C; Plesiat P. Hop Jean Minjoz, Bacteriol Lab, F-25030 Besancon, France (Reprint). PATHOLOGIE BIOLOGIE (OCT 2003) Vol. 51, No. 8-9, pp. 443-448. Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER. 23 RUE LINOIS, 75724 PARIS, FRANCE. ISSN: 0369-8114. Pub. country: France. Language: French.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Exposure of Pseudomonas aeruginosa to aminoglycosides frequently selects for recalcitrant subpopulations exhibiting an unstable, "adaptive" resistance to these antibiotics. In this study, we investigated the implication in the phenomenon of MexXY-OprM, an active efflux system known to export aminoglycosides in P aeruginosa. Immunoblotting experiments demonstrated that the **transporter** MexY, but not the outer membrane pore OprM, was overproduced during the post-drug exposure adaptation period in wild-type strain PAO1. Furthermore, MexY production was dependent upon the degree of bacterial exposure to gentamicin (drug concentration). In contrast to parental strain PAO1, mutants defective in MexXY or in OprM were unable to develop. adaptive resistance. Altogether, these results indicate that the resistance process requires-the rapid production of MexXY and the interaction of these proteins with the constitutively produced component OprM. (C) 2003 Editions scientifiques et medicales Elsevier SAS. Tous droits reserves.

L43 ANSWER 15 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:555360 The Genuine Article (R) Number: 693XW. Efflux-mediated heavy metal resistance in prokaryotes. Nies D H (Reprint). Univ Halle Wittenberg, Inst Microbiol, Kurt Mothes Str 3, D-06099 Halle Saale, Germany (Reprint); Univ Halle Wittenberg, Inst Microbiol, D-06099 Halle Saale, Germany. FEMS MICROBIOLOGY REVIEWS (JUN 2003) Vol. 27, No. 2-3, pp. 313-339. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0168-6445. Pub. country: Germany. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB What makes a heavy metal resistant bacterium heavy metal resistant? The mechanisms of action, physiological functions, and distribution of metal-exporting proteins are outlined, namely: CBA efflux pumps driven by proteins of the resistance-nodulation-cell division superfamily, P-type ATPases, cation diffusion facilitator and chromate proteins, NreB- and CnrT-like resistance factors. The complement of efflux systems of 63 sequenced prokaryotes was compared with that of the heavy metal resistant bacterium Ralstonia metallidurans. This comparison shows that heavy metal resistance is the result of multiple layers of resistance systems with overlapping substrate specificities, but unique functions. Some of these systems are widespread and serve in the basic defense of the cell against superfluous heavy metals, but some are highly specialized and occur only in a few bacteria. Possession of the latter systems makes a bacterium heavy metal resistant. (C) 2003 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

L43 ANSWER 16 OF 37 MEDLINE on STN DUPLICATE 7

2002736582. PubMed ID: 12499164. Contributions of MexAB-OprM and an EmrE homolog to intrinsic resistance of Pseudomonas aeruginosa to aminoglycosides and dyes. Li Xian-Zhi; Poole Keith; Nikaido Hiroshi. (Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3206, USA.) Antimicrobial agents and chemotherapy, (2003 Jan) 47 (1) 27-33. Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB Of the six putative small multidrug resistance (SMR) family proteins of Pseudomonas aeruginosa, a protein encoded by the PA4990 gene (emrE(Pae)) shows the highest identity to the well-characterized EmrE efflux **transporter** of Escherichia coli. Reverse transcription-PCR confirmed the expression of emrE(Pae) in the wild-type strain of P. aeruginosa. Using isogenic emrE(Pae), mexAB-oprM, and/or **mexB** deletion mutants, the contributions of the EmrE protein and the MexAB-OprM efflux system to drug resistance in P. aeruginosa were assessed by a drug susceptibility test carried out in a low-ionic-strength medium, Difco nutrient broth. We found that EmrE(Pae) contributed to intrinsic resistance not only to ethidium bromide and acriflavine but also to aminoglycosides. In this low-ionic-strength medium, MexAB-OprM was also shown to contribute to aminoglycoside resistance, presumably via active efflux. Aminoglycoside resistance caused by these two pumps could not be demonstrated in high-ionic-strength media, such as Luria broth or Mueller-Hinton broth. The EmrE-dependent efflux of ethidium bromide was confirmed by a continuous fluorescence assay.

L43 ANSWER 17 OF 37 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:517134 Document No.: PREV200300519756. Differential impact of **mexB** mutations on the substrate selectivity of the MexAB-OprM multidrug efflux pump of Pseudomonas aeruginosa. Middlemiss, J. K. [Reprint Author]; Poole, K. [Reprint Author]. Queen's University, Kingston, ON, Canada. Abstracts of the General Meeting of the American Society for Microbiology, (2003) Vol. 103, pp. A-148. http://www.asmusa.org/mtgsrc/generalmeeting.htm. cd-rom. Meeting Info.: 103rd American Society for Microbiology General Meeting. Washington, DC, USA. May 18-22, 2003. American Society for Microbiology. ISSN: 1060-2011 (ISSN print). Language: English.

AB Pseudomonas aeruginosa is an opportunistic human pathogen characterized by intrinsic resistance to a broad range of antimicrobial agents. This resistance is provided primarily by MexAB-OprM, a three component multidrug **transporter** of the resistance-nodulation-cell division (RND) family. **MexB**, the integral inner membrane component of this efflux system, consists of twelve transmembrane segments (TMSs), with extensive periplasmic loops occurring between TMS-1 and -2, and TMS-7 and -8. In order to determine the role of **MexB** in substrate recognition and to elucidate the nature of the broad substrate specificity of the MexAB-OprM efflux system, cloned **mexB** was subjected to in vitro hydroxylamine mutagenesis, and mutations impacting antibiotic resistance of a DELTAmexB strain of P. aeruginosa were identified by nucleotide sequencing. Of the ca. four thousand potential mutants screened, approximately one hundred produced wild-type levels of **MexB**, as measured by Western immunoblot, and exhibited increased susceptibility to one or more of a subset of MexAB-OprM antimicrobial substrates, including chloramphenicol, carbenicillin, naladixic acid, novobiocin, aztreonam, meropenem, and piperacillin. Nucleotide sequencing of the **mexB** gene from >30 representative mutants revealed that the majority of amino acid substitutions impacting antibiotic resistance resided within the two substantial periplasmic loops, with a smaller number occurring within the TMSs and loops of the C-terminal region of **MexB**. The location of amino acid substitutions within the three dimensional structure of **MexB** was predicted based upon the crystal structure of the homologous RND **transporter**, AcrB, of Escherichia coli. Mutations in the large periplasmic loops mapped to the pore-forming domain within the lower portion of the **MexB** periplasmic headpiece. Although the mechanism by which these mutations impact substrate selectivity is still unclear, the range of mutations obtained lend support that at least some antimicrobial substates may be recognized differently by the **MexB transporter.**

L43 ANSWER 18 OF 37 MEDLINE on STN DUPLICATE 8
2002666085. PubMed ID: 12426337. Chimeric analysis of the multicomponent multidrug efflux **transporters** from gram-negative bacteria.
Tikhonova Elena B; Wang Quiju; Zgurskaya Helen I. (Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, USA.)
Journal of bacteriology, (2002 Dec) 184 (23) 6499-507. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB Many multidrug **transporters** from gram-negative bacteria belong to the resistance-nodulation-cell division (RND) superfamily of **transporters.** RND-type multidrug **transporters** have an extremely broad substrate specificity and protect bacterial cells from the actions of antibiotics on both sides of the cytoplasmic membrane. They usually function as three-component assemblies spanning the outer and cytoplasmic membranes and the periplasmic space of gram-negative bacteria. The structural determinants of RND **transporters** responsible for multidrug recognition and complex assembly remain unknown. We constructed chimeric RND **transporters** composed of N-terminal residues of AcrB and C-terminal residues of **MexB**, the major RND-type **transporters** from Escherichia coli and Pseudomonas aeruginosa, respectively. The assembly of complexes and multidrug efflux activities of chimeric **transporters** were determined by coexpression of hybrid genes either with AcrA, the periplasmic component of the AcrAB **transporter** from E. coli, or with MexA and OprM, the accessory proteins of the MexAB-OprM pump from P. aeruginosa. We found that the specificity of interaction with the corresponding periplasmic component is encoded in the T60-V612 region of **transporters.** Our results also suggest that the large periplasmic loops of RND-type **transporters** are involved in multidrug recognition and efflux.

L43 ANSWER 19 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2002:519606 The Genuine Article (R) Number: 565ND. Amino acid residues essential for function of the MexF efflux pump protein of Pseudomonas aeruginosa. Aires J R; Pechere J C; Van Delden C; Kohler T (Reprint). CMU, Dept Genet & Microbiol, 9 Av Champel, CH-1211 Geneva 4, Switzerland (Reprint); CMU, Dept Genet & Microbiol, CH-1211 Geneva 4, Switzerland. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY (JUL 2002) Vol. 46, No. 7, pp. 2169-2173. Publisher: AMER SOC MICROBIOLOGY. 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. ISSN: 0066-4804. Pub. country: Switzerland. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB At least four broad-spectrum efflux pumps (Mex) are involved in elevated intrinsic antibiotic resistance as well as in acquired multidrug resistance in Pseudomonas aeruginosa. Substrate specificity of the Mex pumps has been shown to be determined by the cytoplasmic membrane component (**MexB**, MexD, MexF, and MexY) of the tripartite efflux pump system. Alignment of their amino acid sequences with those of the homologous AcrB and AcrD pump proteins of Escherichia coli showed conservation of five charged amino acid residues located in or next to transmembrane segments (TMS). These residues were mutated in the MexF gene by site-directed mutagenesis and replaced by residues of opposite or neutral charge. MexF proteins containing combined D410A and A411G substitutions located in TMS4 were completely inactive. Similarly, the substitutions E417K (next to TMS4) and K951E (TMS10) also caused loss of activity towards all tested antibiotics. The substitution E349K in TMS2 resulted in a MexF mutant protein which was unable to transport trimethoprim and quinolones but retained partial activity for the transport of chloramphenicol. All mutated MexF proteins were expressed at comparable levels when tested by Western blot analysis. It is concluded that charged residues located in or close to TMS are essential for proper function of MexF.

L43 ANSWER 20 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2003:17141 The Genuine Article (R) Number: 623BQ. Regulation of bacterial drug export systems. Grkovic S; Brown M H; Skurray R A (Reprint). Univ Sydney, Sch Biol Sci, Macleay Bldg A12, Sydney, NSW 2006, Australia (Reprint); Univ Sydney, Sch Biol Sci, Sydney, NSW 2006, Australia. MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS (DEC 2002) Vol. 66, No. 4, pp. 671-+. Publisher: AMER SOC MICROBIOLOGY. 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. ISSN: 1092-2172. Pub. country: Australia. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The active transport of toxic compounds by membrane-bound efflux proteins is becoming an increasingly frequent mechanism by which cells exhibit resistance to therapeutic drugs. This review examines the regulation of bacterial drug efflux systems, which occurs primarily at the level of transcription. Investigations into these regulatory networks have yielded a substantial volume of information that has either not been forthcoming from or complements that obtained by analysis of the transport proteins themselves. Several local regulatory proteins, including the activator BmrR from Bacillus subtilis and the repressors QacR from Staphylococcus aureus and TetR and EmrR from Escherichia coli, have been shown to mediate increases in the expression of drug efflux genes by directly sensing the presence of the toxic substrates exported by their cognate pump. This ability to bind **transporter** substrates has permitted detailed structural information to be gathered on protein-antimicrobial agent-ligand interactions. In addition, bacterial multidrug efflux determinants are frequently controlled at a global level and may belong to stress response regulons such as E coli mar, expression of which is controlled by the MarA and MarR proteins. However, many regulatory systems are ill-adapted for detecting the presence of toxic pump substrates and instead are likely to respond to alternative signals related to unidentified physiological roles of the **transporter.** Hence, in a number of important pathogens, regulatory mutations that result in drug **transporter** overexpression and concomitant elevated antimicrobial resistance are often observed.

L43 ANSWER 21 OF 37 MEDLINE on STN DUPLICATE 9
2002174355. PubMed ID: 11906191. Secondary-site mutation restores the transport defect caused by the transmembrane domain mutation of the xenobiotic **transporter MexB** in Pseudomonas aeruginosa. Yoneyama Hiroshi; Maseda Hideaki; Yamabayashi Ta Taka-aki; Izumi Shotaro; Nakae Taiji. (Department of Molecular Life Science, Tokai University School of Medicine, Isehara, 259-1193, Japan.) Biochemical and biophysical research communications, (2002 Mar 29) 292 (2) 513-8. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB It has been suggested that the **MexB** subunit of the MexAB-OprM efflux **transporter** of Pseudomonas aeruginosa exports xenobiotics in an energy-dependent manner. To investigate the role of the transmembrane segments (TMS) of **MexB** in the **transporter** activity, we isolated 24 spontaneous mutants showing hypersusceptibility to antibiotics. Among them, three mutations were located at TMS-3, TMS-4, and TMS-10 having amino acid substitution Leu376vPro, Gly397vVal, and Val928vGly, respectively. A secondary mutation, which suppressed the defect caused by the Val928vGly mutation in TMS-10, was found at the 403rd amino acid residue in TMS-4 with a change of glycine to serine, suggesting that TMS-4 and TMS-10 may be in close proximity. This result provided strong support for the recent notion that negatively charged residues in TMS-4 might form a salt-bridge with a positive charge in TMS-10 (Guan, L., and Nakae, T. (2001) J. Bacteriol. 183, 1734-1739). The **transporter** function impaired by the Gly397vVal mutation in TMS-4 was recovered by the secondary mutation, Gln998vHis, in the loop between

TMS-11 and TMS-12, thereby suggesting that TMS-4 and TMS-11 or TMS-12 might also be in close proximity. Thus, it is most likely that TMS-4, TMS-10, and TMS-11 or TMS-12 are packed close three dimensionally. (c)2002 Elsevier Science (USA).

L43 ANSWER 22 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:977140 The Genuine Article (R) Number: 620CN. The substrate specificity of tripartite efflux systems of Pseudomonas aeruginosa is determined by the RND component. Murata T; Kuwagaki M; Shin T; Gotoh N (Reprint); Nishino T. Kyoto Pharmaceut Univ, Dept Microbiol, Kyoto 6078414, Japan (Reprint); Osaka Univ, Inst Sci & Ind Res, Dept Nanosyst Design, Ibaraki, Osaka 5670047, Japan. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (29 NOV 2002) Vol. 299, No. 2, pp. 247-251. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0006-291X. Pub. country: Japan. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The tripartite efflux systems MexAB-OprM and MexCD-OprJ of Pseudomonas aeruginosa each display characteristic substrate specificity against a variety of antimicrobial agents. The chimeric efflux system MexC-**MexB**-OprJ/DeltaMexD constructed by exchange of MexD with **MexB** endowed the recombinant host the same resistance profile as MexAB-OprM rather than MexCD-OprJ. The change of substrate specificity vas shown to be due to extrusion from the chimeric efflux system by cellular accumulation experiments using tetracycline, erythromycin, and ethidium bromide. Thus, we conclude that **MexB** and MexD are primary components of the efflux system responsible for sorting extrusion substrates. (C) 2002 Elsevier Science (USA). All rights reserved.

L43 ANSWER 23 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:300147 The Genuine Article (R) Number: 535JF. Direct measurement of sizes and dynamics of single living membrane **transporters** using nanooptics. Xu X H N (Reprint); Chen J; Jeffers R B; Kyriacou S. Old Dominion Univ, Dept Chem & Biochem, Norfolk, VA 23529 USA (Reprint). NANO LETTERS (MAR 2002) Vol. 2, No. 3, pp. 175-182. Publisher: AMER CHEMICAL SOC. 1155 16TH ST, NW, WASHINGTON, DC 20036 USA. ISSN: 1530-6984. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Sizes and dynamics of single membrane **transporters** on single living cells, Pseudomonas aeruginosa, were directly measured showing transported substrates up to 80 nm in diameter, which is one order magnitude larger than the reported exclusion limit of the outer membrane, over times of seconds to hours. The uptake and efflux dynamics depend on size and concentration of substrates, incubation time, and mutants. The strikingly active efflux of substrates by mutants devoid of pump proteins was observed, suggesting that substrates trigger the assembly of a new extrusive system. This new observation is in excellent agreement with the recent results of genome project analysis showing that P. aeruginosa encode more than a dozen unidentified efflux pump proteins. An extraordinary heterogeneity of size and dynamics of membrane transport mechanisms was observed, demonstrating real-time transformation of membrane permeability and an active extrusion system. This constitutes the first direct observation of living membrane transportation triggered by substrates with a variety of sizes at the single membrane pump level and opens up the new possibility of direct measurements of membrane active extrusion mechanisms for advancing the understanding of multidrug resistance and living molecular pumps.

L43 ANSWER 24 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:685456 The Genuine Article (R) Number: 583KD. Molecular analysis of efflux pump-based antibiotic resistance. Zgurskaya H I (Reprint). Univ Oklahoma, Dept Chem & Biochem, 620 Parrington Oval, Room 208, Norman, OK 73019 USA (Reprint); Univ Oklahoma, Dept Chem & Biochem, Norman, OK 73019

USA. INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY (JUL 2002) Vol. 292, No. 2, pp. 95-105. Publisher: URBAN & FISCHER VERLAG. BRANCH OFFICE JENA, P O BOX 100537, D-07705 JENA, GERMANY. ISSN: 1438-4221. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Multidrug efflux **transporters** are normal constituents of bacterial cells. These **transporters** are major contributors to intrinsic resistance of bacteria to many anti-microbial agents. In clinical settings, exposure to antibiotics promotes the mutational overexpression of active or silent multidrug **transporters**, leading to increased antibiotic resistance without acquisition of multiple, specific resistance determinants. The paradoxical ability of multidrug **transporters** to recognize and efficiently expel from cells scores of dissimilar organic compounds has been in the focus of extensive research for many years. Several independent studies implied that the mechanistic basis of such ability lies in a distinctive locus of the **transporter**-substrate interaction: the multidrug **transporters** select and bind their substrates within the phospholipid bilayer. The recently reported high-resolution structure of a complete MsbA **transporter** of Escherichia coli provides a solid structural basis for these studies. Although the majority of multidrug **transporters** function as single-component pumps, major **transporters** of Gram-negative bacteria are organized as three-component structures. Special outer membrane channels and periplasmic proteins belonging to the membrane fusion protein family enable drug efflux across a Gram-negative two-membrane envelope, directly into the external medium. This ninireview focuses on the current status of research in the field of multidrug efflux mechanisms.

L43 ANSWER 25 OF 37 MEDLINE on STN DUPLICATE 10
2001181780. PubMed ID: 11160105. Identification of essential charged residues in transmembrane segments of the multidrug **transporter** **MexB** of Pseudomonas aeruginosa. Guan L; Nakae T. (Department of Molecular Life Science, Tokai University School of Medicine, Isehara 259-1193, Japan.) Journal of bacteriology, (2001 Mar) 183 (5) 1734-9. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB The MexABM efflux pump exports structurally diverse xenobiotics, utilizing the proton electrochemical gradient to confer drug resistance on Pseudomonas aeruginosa. The **MexB** subunit traverses the inner membrane 12 times and has two, two, and one charged residues in putative transmembrane segments 2 (TMS-2), TMS-4, and TMS-10, respectively. All five residues were mutated, and **MexB** function was evaluated by determining the MICs of antibiotics and fluorescent dye efflux. Replacement of Lys342 with Ala, Arg, or Glu and Glu346 with Ala, Gln, or Asp in TMS-2 did not have a discernible effect. Ala, Asn, or Lys substitution for Asp407 in TMS-4, which is well conserved, led to loss of activity. Moreover, a mutant with Glu in place of Asp407 exhibited only marginal function, suggesting that the length of the side chain at this position is important. The only replacements for Asp408 in TMS-4 or Lys939 in TMS-10 that exhibited significant function were Glu and Arg, respectively, suggesting that the native charge at these positions is required. In addition, double neutral mutants or mutants in which the charged residues Asp407 and Lys939 or Asp408 and Lys939 were interchanged completely lost function. An Asp408-->Glu/Lys939-->Arg mutant retained significant activity, while an Asp407-->Glu/Lys939-->Arg mutant exhibited only marginal function. An Asp407-->Glu/Asp408-->Glu double mutant also lost activity, but significant function was restored by replacing Lys939 with Arg (Asp407-->Glu/Asp408-->Glu/Lys939-->Arg). Taken as a whole, the findings indicate that Asp407, Asp408, and Lys939 are functionally important and raise the possibility that Asp407, Asp408, and Lys939 may form a charge network between TMS-4 and TMS-10 that is important for proton translocation and/or energy coupling.

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on STN
2001:864751 The Genuine Article (R) Number: 487EP. Multidrug resistance in Gram-negative bacteria. Poole K (Reprint). Queens Univ, Dept Microbiol & Immunol, Kingston, ON K7L 3N6, Canada (Reprint). CURRENT OPINION IN MICROBIOLOGY (OCT 2001) Vol. 4, No. 5, pp. 500-508. Publisher: CURRENT BIOLOGY LTD. 84 THEOBALDS RD, LONDON WC1X 8RR, ENGLAND. ISSN: 1369-5274. Pub. country: Canada. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Broadly specific, so-called multidrug, efflux mechanisms are now known to contribute significantly to intrinsic and acquired multidrug resistance in a number of Gram-negative bacteria, and the boom in bacterial genomics has confirmed the distribution of these systems in all bacteria. This broad distribution, of multidrug **transporters** lends a certain credibility to suggestions that they play a housekeeping role in the cell, beyond any contributions they may make to antimicrobial efflux and resistance. In many instances, these **transporters** are dispensable, arguing against their carrying out essential cellular functions; nevertheless, the multiplicity of these broadly specific export systems within a given microorganism, often with overlapping substrate specificity, may explain the dispensability of individual exporters. Whatever their intended function, however, their conservation in so many organisms highlights their probable general importance in antimicrobial resistance, particularly in Gram-negative bacteria whose outer membranes work synergistically with many of these export systems to promote drug exclusion.

L43 ANSWER 27 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2001:591030 The Genuine Article (R) Number: 452LM. Transcription regulation of multidrug efflux pumps in bacteria. Grkovic S; Brown M H; Skurray R A (Reprint). Univ Sydney, Sch Biol Sci, Macleay Bldg A12, Sydney, NSW 2006, Australia (Reprint); Univ Sydney, Sch Biol Sci, Sydney, NSW 2006, Australia. SEMINARS IN CELL & DEVELOPMENTAL BIOLOGY (JUN 2001) Vol. 12, No. 3, pp. 225-237. Publisher: ACADEMIC PRESS LTD. 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND. ISSN: 1084-9521. Pub. country: Australia. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB As integral membrane proteins demonstrating an extraordinarily wide substrate range, some degree of regulatory control over the expression of bacterial multidrug-resistance (MDR) **transporters** is to be expected. Excessive expression could be deleterious, due to direct, physical disruption of membrane integrity, or the unwanted export of essential metabolites, a potential side-effect of their broad substrate specificity. There are limited clues as the the physiological functions for the most MDR **transporters**, but their expression is likely to be upregulated in response to the presence of natural substrates of these pumps. Thus, it is no surprise that MDR genes are subject to regulation at the local level, consisting of examples of both transcriptional repression and activation by proteins encoded adjacent to that for the **transporter.** Furthermore, an increasing number of MDR genes have also been found to be controlled by global transcriptional activator proteins.

L43 ANSWER 28 OF 37 MEDLINE on STN
2001505482. PubMed ID: 11347972. Microbial fermentation-derived inhibitors of efflux-pump-mediated drug resistance. Lee M D; Galazzo J L; Staley A L; Lee J C; Warren M S; Fuernkranz H; Chamberland S; Lomovskaya O; Miller G H. (Microcide Pharmaceuticals, Inc., Mountain View, CA 94043, USA.. mlee@microcide.com) . Farmaco (Societa chimica italiana : 1989), (2001 Jan-Feb) 56 (1-2) 81-5. Ref: 15. Journal code: 8912641. ISSN: 0014-827X. Pub. country: Italy. Language: English.

AB A library of 85000 microbial fermentation extracts was screened for inhibitors of multidrug resistance efflux pumps in Pseudomonas aeruginosa and Candida albicans. New compounds EA-371alpha and EA-371delta were isolated and demonstrated to be potent and specific inhibitors of the

MexAB-OprM pump in P. aeruginosa. Two series of fungal metabolites, enniatins and beauvericins, were found to be ubiquitous and potent inhibitors of ABC **transporters.** Milbemycins were rediscovered as potent inhibitors of the CDRI pump in C. albicans, and demonstrated to potentiate effectively the antifungal activity of fluconazole and SCH-56592 against a wide variety of Candida clinical isolates.

L43 ANSWER 29 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN
2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L43 ANSWER 30 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2001:185969 The Genuine Article (R) Number: 403RN. Molecular properties of bacterial multidrug **transporters.** Putman M; van Veen H W; Konings W N (Reprint). Univ Groningen, Groningen Biomol Sci & Biotechnol Inst, Dept Microbiol, Kerklaan 30, NL-9751 NN Haren, Netherlands (Reprint); Univ Groningen, Groningen Biomol Sci & Biotechnol Inst, Dept Microbiol, NL-9751 NN Haren, Netherlands. MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS (DEC 2000) Vol. 64, No. 4, pp. 672-+. Publisher: AMER SOC MICROBIOLOGY. 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. ISSN: 1092-2172 . Pub. country: Netherlands. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB One of the mechanisms that bacteria utilize to evade the toxic effects of antibiotics is the active extrusion of structurally unrelated drugs from the cell. Both intrinsic and acquired multidrug **transporters** play an important role in antibiotic resistance of several pathogens, including Neisseria gonorrhoeae, Mycobacterium tuberculosis, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa and Vibrio cholerae. Detailed knowledge of the molecular basis of drug recognition and transport by multidrug transport systems is required for the development of new antibiotics that are not extruded ol of inhibitors which block the multidrug **transporter** and allow traditional antibiotics to be effective. This review gives an extensive overview of the currently known multidrug **transporters** in bacteria. Based on energetics and structural characteristics, the bacterial multidrug **transporters** can be classified into five distinct families. Functional reconstitution in liposomes of purified multidrug transport proteins from four families revealed that these proteins are capable of mediating the export of structurally unrelated drugs independent of accessory proteins or cytoplasmic components. On the basis of (i) mutations that affect the activity or the substrate specificity of multidrug **transporters** and (ii) the three-dimensional structure of the drug-binding domain of the laboratory protein BmrR, the substrate-binding site for cationic drugs is predicted to consist of a hydrophobic pocket with a buried negatively charged residue that interacts electrostatically with the positively charged substrate. The aromatic and hydrophobic amino acid residues which form the drug-binding pocket impose restrictions on the shape and size of the substrates. Kinetic analysis of drug transport by multidrug **transporters** provided evidence that these proteins may contain multiple substrate-binding sites.

L43 ANSWER 31 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2000:564492 The Genuine Article (R) Number: 335GE. Antibiotic efflux pumps - Commentary. VanBambeke F (Reprint); Balzi E; Tulkens P M. UNIV CATHOLIQUE LOUVAIN, UNITE PHARMACOL CELLULAIRE & MOL, 73-70 AVE E MOUNTER 73, B-1200 BRUSSELS, BELGIUM (Reprint); UNIV CATHOLIQUE LOUVAIN, UNITE BIOCHIM PHYSIOL, B-1348 LOUVAIN, BELGIUM. BIOCHEMICAL PHARMACOLOGY (15 AUG 2000) Vol. 60, No. 4, pp. 457-470. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0006-2952. Pub. country: BELGIUM. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Active efflux from procaryotic as well as eucaryotic cells strongly modulates the activity of a large number of antibiotics. Effective antibiotic transport has now been observed for many classes of drug efflux pumps. Thus, within the group of primary active **transporters**, predominant in eucaryotes, six families belonging to the ATP-binding cassette superfamily, and including the P-glycoprotein in the MDR (Multi Drug Resistance) group and the MRP (Multidrug Resistance protein), have been recognized as being responsible for antibiotic efflux. Within the class of secondary active **transporters** (antiports, symports, and uniports), ten families of antibiotic efflux pill-ups have been described, distributed in five superfamilies [SMR (Small Multidrug Resistance), MET (Multidrug Endosomal **Transporter**), MAR (Multi Antimicrobial Resistance), RND (Resistance Nodulation Division), and MFS (Major Facilitator Superfamily)]. Nowadays antibiotic efflux pumps are believed to contribute significantly to acquired bacterial resistance because of the very broad variety of substrates they recognize, their expression in important pathogens, and their cooperation with other mechanisms of resistance. Their presence also explains high-level intrinsic resistances found in specific organisms. Stable mutations in regulatory genes can produce phenotypes of irreversible multidrug resistance. In eucaryotes, antibiotic efflux pumps modulate the accumulation of antimicrobials in phagocytic cells and play major roles in their transepithelial transport. The existence of antibiotic efflux pumps, and their impact on therapy, must now be taken fully into account for the selection of novel antimicrobials. The design of specific, potent inhibitors appears to be an important goal for the improved control of infectious diseases in the near future. BIOCHEM PHARMACOL 60;4:457-470, 2000. (C) 2000 Elsevier Science Inc.

L43 ANSWER 32 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2000:569082 The Genuine Article (R) Number: 336PN. Multidrug resistance mechanisms: drug efflux across two membranes. Zgurskaya H I; Nikaido H (Reprint). UNIV CALIF BERKELEY, DEPT MOL & CELL BIOL, BERKELEY, CA 94720 (Reprint); UNIV CALIF BERKELEY, DEPT MOL & CELL BIOL, BERKELEY, CA 94720. MOLECULAR MICROBIOLOGY (JUL 2000) Vol. 37, No. 2, pp. 219-225. Publisher: BLACKWELL SCIENCE LTD. P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND. ISSN: 0950-382X. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A set of multidrug efflux systems enables Gram-negative bacteria to survive in a hostile environment. This review focuses on the structural features and the mechanism of major efflux pumps of Gram-negative bacteria, which expel from the cells a remarkably broad range of antimicrobial compounds and produce the characteristic intrinsic resistance of these bacteria to antibiotics, detergents, dyes and organic solvents. Each efflux pump consists of three components: the inner membrane **transporter**, the outer membrane channel and the periplasmic lipoprotein. Similar to the multidrug **transporters** from eukaryotic cells and Gram-positive bacteria, the inner membrane **transporters** from Gram-negative bacteria recognize and expel their substrates often from within the phospholipid bilayer. This efflux occurs without drug accumulation in the periplasm, implying that substrates are pumped out across the two membranes directly into the medium. Recent data suggest that the molecular mechanism of the drug extrusion across a two-membrane envelope of Gram-negative bacteria may involve the formation of the membrane adhesion sites between the inner and the outer membranes. The periplasmic components of these pumps are proposed to cause a close membrane apposition as the complexes are assembled for the transport.

L43 ANSWER 33 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN
1999:734683 Document No. 132:216310 Resistance to macrolides mediated by efflux mechanisms. Sutcliffe, Joyce (Department of Infectious Diseases, Pfizer Central Research Inc, Groton, CT, 06340, USA). Current Opinion in Anti-Infective Investigational Drugs, 1(4), 403-412 (English) 1999. CODEN: COADFY. ISSN: 1464-8458. Publisher: Current Drugs Ltd..

AB A review with 92 refs. Drug transport proteins can be divided into two classes: secondary **transporters**, which couple extrusion of drugs with ion exchange, and ATP-binding cassette (ABC) **transporters**, which utilize the energy of a phosphate bond upon ATP hydrolysis to efflux drugs. Efflux is mediated by either a pump with narrow specificity, e.g, Mef and MsrA, or a pump with broad specificity, e.g., AcrAB-TolC, AcrEF-TolC, MexAB-OprM, MexCD-OprJ, AmrAB-OprA, MtrCDE, EmrE, MdfA and Cmr. Staphylococcus epidermidis harbors a plasmid with an msrA determinant, an ABC-type **transporter** specific for 14- and 15-membered macrolides and streptogramin B. An almost identical gene resides in Staphylococcus aureus (S aureus), although it appears to be uncommon. MsrA appears to be the ATP binding component of the ABC **transporter** and likely works in concert with various pumps that have been identified on plasmids or on the chromosome of staphylococcal strains. MsrB, found in Staphylococcus xylosus, is identical to the C-terminal portion of MsrA and likely functions as a dimer. MefA and MefE, initially described in Streptococcus pyogenes and Streptococcus pneumoniae, resp., have been folded into a single family, MefA, because the two genes are approx. 90% identical at the amino acid level. The MefA protein is a member of the major facilitator superfamily (MFS) and can efflux 14- and 15- membered macrolides. Resistance to 16-membered macrolides is not seen even in the presence of inducing concns. of erythromycin. Strains harboring the mefA gene remain susceptible to streptogramin B and clindamycin. Interestingly, the MefA protein conferred macrolide resistance when cloned into Escherichia coli (E coli). More recently, mefA has been described in many different streptococcal species, moderately erythromycin-resistant strains of Enterococcus faecium, Micrococcus luteus and Corynebacterium jeikeiium. Other members of the MFS class that can cause the active egress of erythromycin include MdfA in E coli and Cmr in Corynebacterium glutamicum. The latter two require overexpression to confer approx. 4-fold level of resistance. Another putative pump, MreA, was described in a clin. strain of Streptococcus agalactiae, but recent work demonstrates that this protein likely encodes a riboflavin kinase (ribC). The AcrAB-TolC pump is found in E coli and similar members of the resistance-nodulation-cell division family appear to be present in Haemophilus influenzae (AcrAB-like), Pseudomonas aeruginosa (MexAB-OprM, MexCD-OprJ), Burkholderia pseudomallei (AmrAB-OprA) and Neisseria gonorrhoeae (MtrCDE). The **transporters** in the Gram-neg. bacteria (AcrB, AcrF, **MexB**, MexD, AmrB, MtrD) work in concert with an accessory protein (AcrA, AcrE, MexA, MexC, AmrA, MtrC) which spans the periplasmic space and interacts with a porin in the outer membrane (TolC, TolC(), OprM, OprJ, OprA, MtrE). AcrAB-TolC and MtrCDE confer resistance to hydrophobic antibiotics (including erythromycin), detergents and dyes, while the two multidrug pumps from Pseudomonas overlap in their ability to efflux compds. such as tetracycline, novobiocin, chloramphenicol, erythromycin and fluoroquinolones, but show specificity toward certain β-lactams and fourth generation cephalosporins. AmrAB-OprA confers high-level resistance to aminoglycosides and macrolides. Some of these pumps are constitutive, e.g., AcrAB-TolC, AcrAB-Omp2, MexAB-OprM, AmrAB-OprA, while others remain silent until promoter or regulatory mutations allow their expression. Macrolide resistance can also be conferred by members of the small multidrug resistance family, EmrE and Mmr. EmrE is chromosomally encoded in E coli and has no known transmembrane linker or outer membrane channel associated with it. Reconstituted EmrE catalyzes the efflux or erythromycin and tetracycline, in addition to lipophilic monovalent cations. Mmr has been cloned from Mycobacterium tuberculosis and confers erythromycin resistance in Mycobacterium smegmatis. All the resistance determinants are intrinsic and chromosomally encoded, with the exception of msrA and mefA. Recently, mefA was transferred to naive strains by conjugation; it is likely mobile by a chromosomally integrated transposon. MsrA is generally plasmid-mediated. The expression of both genes is inducible by erythromycin.

L43 ANSWER 34 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
1999:336988 The Genuine Article (R) Number: 189TM. Mechanisms of fluoroquinolone resistance. Hooper D C (Reprint). HARVARD UNIV, DIV INFECT DIS, MASSACHUSETTS GEN HOSP, SCH MED, 55 FRUIT ST, BOSTON, MA 02114 (Reprint). DRUG RESISTANCE UPDATES (APR 1999) Vol. 2, No. 1, pp. 38-55. Publisher: CHURCHILL LIVINGSTONE. JOURNAL PRODUCTION DEPT, ROBERT STEVENSON HOUSE, 1-3 BAXTERS PLACE, LEITH WALK, EDINBURGH EH1 3AF, MIDLOTHIAN, SCOTLAND. ISSN: 1368-7646. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Mechanisms of bacterial resistance to fluoroquinolones fall into two principal categories, alterations in drug target enzymes and alterations that limit permeation of drug to the target, both resulting from chromosomal mutations. No specific resistance mechanisms of quinolone degradation or modification have been found. The target enzymes, DNA gyrase and topoisomerase IV are most commonly altered in domains near the enzyme active sites and in some cases reduced drug binding affinity has been demonstrated. Drug permeation is altered by mutations that increase expression of endogenous multidrug efflux pumps, alter outer membrane diffusion channels, or both. Recently a new plasmid-mediated resistance of an as yet undefined mechanism was found in clinical isolates of Klebsiella pneumoniae.

L43 ANSWER 35 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
1998:817399 The Genuine Article (R) Number: 130LP. Multiple antibiotic resistance and efflux. Nikaido H (Reprint). UNIV CALIF BERKELEY, DEPT MOL & CELL BIOL, 229 STANLEY HALL, BERKELEY, CA 94720 (Reprint). CURRENT OPINION IN MICROBIOLOGY (OCT 1998) Vol. 1, No. 5, pp. 516-523. Publisher: CURRENT BIOLOGY LTD. 34-42 CLEVELAND STREET, LONDON W1P 6LB, ENGLAND. ISSN: 1369-5274. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Multiple antibiotic resistance in bacteria was at first thought to be caused exclusively by the combination of several resistance genes, each coding for resistance to a single drug, More recently, it became clear that such phenotypes are often achieved by the activity of drug efflux pumps. Some of these efflux pumps exhibit an extremely wide specificity covering practically all antibiotics, chemotherapeutic agents, detergents, dyes, and other inhibitors, the exception perhaps being very hydrophilic compounds. Such efflux pumps work with exceptional efficiency in Gram-negative bacteria through their synergistic interaction with the outer membrane barrier. It is disturbing that the antibacterial agents of the most advanced type, which are unaffected by common resistance mechanisms, are precisely the compounds whose use appears to select for multidrug-resistant mutants that overproduce these efflux pumps of wide specificity.

L43 ANSWER 36 OF 37 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 11
1998113406 EMBASE [Active efflux systems in pseudomonas aeruginosa]. SYSTEMES D'EFFLUX ACTIFS CHEZ PSEUDOMONAS AERUGINOSA. Plesiat P.; Ramos-Aires J.;

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     Pechere J.-C.; Kohler T.. P. Plesiat, Laboratoire de Bacteriologie,
     Faculte de Medecine, Besancon, France. Medecine et Maladies Infectieuses
     28/SPEC. ISS. FEB. (126-133)  1998.
     Refs: 42.
     ISSN: 0399-077X. CODEN: MMAIB5. Pub. Country: France. Language: French.
     Summary Language: English; French.
AB   Three active efflux systems have been described in Pseudomonas aeruginosa:
     namely MexA-MexB-OprM, MexC-MexD-OprJ, and MexE-MexF-OprN. Each
     of these systems consists of 3 proteins, one of which is a
     broadly-specific transporter able to accommodate a variety of
     amphiphilic substrates. Over- expression of the efflux system, consecutive
     to mutations in regulator genes, enhances the resistance levels of the
     organism (MICs x 4 to 8-fold) to structurally unrelated antibiotics such
     as quinolones, tetracyclines, chloramphenicol, trimethoprim, and various
     β-lactams. Therapeutical impact of multidrug resistance remains to be
     investigated.

L43  ANSWER 37 OF 37  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared


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L44          67 L11 AND "SMR"

=> dup remove l44
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L45  ANSWER 1 OF 26  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN                                                   DUPLICATE 1
2005014190  EMBASE  [Efflux-mediated antibiotics resistance in bacteria].
     POMPES D'EFFLUX ET RESISTANCE AUX ANTIBIOTIQUES CHEZ LES BACTERIES.
     Cattoir V.. . Vincent.Cattoir@chu-rennes.fr. Pathologie Biologie  52/10
     SPEC. ISS. (607-616)  2004.
     Refs: 30.
     ISSN: 0369-8114. CODEN: PTBIAN.
     Publisher Ident.: S 0369-8114(04)00204-4. Pub. Country: France. Language:
     French. Summary Language: English; French.
AB   Bacteria can resist to antibiotics by active exportation mediated by
     membrane transporters called efflux pumps. These proteins can be
     specific of a class of antibiotics or responsible for multidrug resistance
     (MDR). Energy required by efflux pumps can be provided by transmembrane
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electrochemical gradient of protons (MFS, RND, **SMR** families) or sodium ions (MATE family) or by ATP hydrolysis (ABC family). Several physiological functions have been described in prokaryotes, such as protection from environmental toxics and regulation of cell homeostasis, which can indirectly contributes to bacterial virulence. In Gram-negative bacteria, efflux **transporters** usually are organized as multicomponent systems in wich the efflux pump located in the inner membrane works in conjunction with a periplasmic fusion protein and an outer membrane factor. The most frequently encountered pumps are of the RND-type such as AcrB in Escherichia coli or MexB in Pseudomonas aeruginosa. In Gram-positive bacteria, efflux is solely mediated by the pump protein, so described with MFS pumps such as NorA or QacA in Staphylococcus aureus and PmrA in Streptococcus pneumoniae. Efflux **transporters** have also been described in mycobacteria. Although numerous bacterial pumps have been characterized, the clinical consequences of efflux-mediated resistance are mostly unknown because of variable levels of expression and of the lack of specific markers in laboratory practice. Finally, associating pump-specific inhibitors to efflux-sensitive antibiotics might prove an interesting therapeutic perspective. However, inhibitors that are not toxic to eukaryotic cells remain to be identified. .COPYRGT. 2004 Elsevier SAS. Tous droits reserves.

L45 ANSWER 2 OF 26 CAPLUS COPYRIGHT 2005 ACS on STN
2004:173498 Document No. 141:34383 Insight into the genome of Aspergillus fumigatus: analysis of a 922 kb region encompassing the nitrate assimilation gene cluster. Pain, Arnab; Woodward, John; Quail, Michael A.; Anderson, Michael J.; Clark, Richard; Collins, Matthew; Fosker, Nigel; Fraser, Audrey; Harris, David; Larke, Natasha; Murphy, Lee; Humphray, Sean; O'Neil, Susan; Pertea, Mihaela; Price, Claire; Rabbinowitsch, Ester; Rajandream, Marie-Adele; Salzberg, Steven; Saunders, David; Seeger, Kathy; Sharp, Sarah; Warren, Tim; Denning, David W.; Barrell, Bart; Hall, Neil (The Pathogen Sequencing Unit, The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK). Fungal Genetics and Biology, 41(4), 443-453 (English) 2004. CODEN: FGBIFV. ISSN: 1087-1845. Publisher: Elsevier.

AB Aspergillus fumigatus is the most ubiquitous opportunistic filamentous fungal pathogen of human. As an initial step toward sequencing the entire genome of A. fumigatus, which is estimated to be .apprx.30 Mb in size, a 922-kb region, contained within 16 overlapping bacterial artificial chromosome (BAC) clones, was sequenced. Fifty-four percent of the DNA is predicted to be coding with 341 putative protein coding genes. Functional classification of the proteins showed the presence of a higher proportion of enzymes and membrane **transporters** when compared to those of Saccharomyces cerevisiae. In addition to the nitrate assimilation gene cluster, the quinate utilization gene cluster is also present on this 922-kb genomic sequence. Large scale synteny between A. fumigatus and Aspergillus nidulans was observed by comparing this sequence to the A. nidulans genetic map of linkage group VIII.

L45 ANSWER 3 OF 26 CAPLUS COPYRIGHT 2005 ACS on STN
2004:919884 Genomic and structural diversity of antiseptic resistance gene **smr** encoding multidrug efflux protein in staphylococci. Kobayashi, Nobumichi; Alam, Mohammed Mahbub; Ishino, Masaho; Mise, Keiji (Department of Hygiene, Sapporo Medical University School of Medicine, Chuo-ku, Sapporo, 060-8556, Japan). Drug Design Reviews--Online, 1(4), 303-312 (English) 2004. CODEN: DDRRAM. URL: http://www.ingenta.com/isis/browsing/TOC/ingenta;jsessionid=1cwq23yrg8ba7.crescent?issue=pubinforbike://ben/cdd/2004/00000001/00000004 Publisher: Bentham Science Publishers Ltd..

AB Antiseptic resistance of staphylococci is caused by multidrug **transporter** proteins represented by QacA/QacB and **Smr** /QacC which mediate efflux of drugs via electrochem. proton gradient across the cytoplasmic membrane. **Smr**/QacC is a member of small multidrug resistance (**SMR**) family which consists of approx. 110

amino acids and contains four predicted transmembrane segments. **SMR** family includes various antiseptic resistance proteins which have been found in staphylococci as well as in other bacterial species. Phylogenetically. **SMR** family proteins are classified into two major clusters, genogroup 1 and genogroup 2, which are mostly found in Gram-pos. cocci and Gram-neg. bacteria, resp. Although amino acid sequences of **SMR** family are divergent among different genotypes, several amino acids in transmembrane regions are highly conserved and considered to be directly associated with a process of drug transport through the bacterial cytoplasmic membrane. The **Smr**/QacC which is encoded by **smr** is genetically well conserved in staphylococci. However, genomic diversity is found outside the ORF of **smr** gene and three types of **smr** gene cassette which is a structural unit containing **smr** and terminal direct repeats (DRs) have been discriminated. Although antiseptic resistance levels of Staphylococcus aureus having **smr** in each of the three types of cassettes were generally similar, higher copy nos. were detected for type 2 cassette-**smr** than type 1 cassette-**smr** in our recent study, suggesting that expression level of **smr** might be different depending on the type of **smr** gene cassette. Further study of genomic structure and diversity of **smr** gene will contribute to prevent spread of antiseptic resistance in staphylococci and development of novel antiseptic substances.

L45 ANSWER 4 OF 26 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:124007 Document No.: PREV200400127060. Studies of the refolding behaviour of small multidrug resistance proteins. Charalambous, Kalypso N. [Reprint Author]; Curnow, Paul [Reprint Author]; Booth, Paula Jane [Reprint Author]. Biochemistry, University of Bristol, Bristol, UK. Biophysical Journal, (January 2004) Vol. 86, No. 1, pp. 260a. print.
Meeting Info.: 48th Annual Meeting of the Biophysical Society. Baltimore, MD, USA. February 14-18, 2004. Biophysical Society.
ISSN: 0006-3495 (ISSN print). Language: English.

AB The understanding of the mechanism by which integral membrane proteins undergo folding at the molecular level has progressed greatly due to the use of phosphatidylcholine lipid bilayers as model membranes. This approach has been successfully applied to a number of proteins, including Bacteriorhodopsin and E. coli diacylglycerol kinase. This investigation utilises this approach for the detailed study of multidrug **transporters** such as the small multidrug resistance (**smr**) family. These proteins enable bacteria to remove toxins from the cytoplasm, which has led to the evolution of multidrug resistant bacteria. Here we present a study of the refolding behaviour in vitro of 3 **smr** proteins; E. coli multidrug resistance enzyme (EmrE), Mycobacterium tuberculosis **smr** (TBsmr) and Pseudomonas aureginosa **smr** (PAsmr) using a novel transport assay and kinetic analysis in an attempt to further the understanding of the mechanisms that govern the dynamics of this important class of proteins.

L45 ANSWER 5 OF 26 MEDLINE on STN DUPLICATE 2

2004151436. PubMed ID: 15044024. The membrane topology of EmrE - a small multidrug **transporter** from Escherichia coli. Ninio Shira; Elbaz Yael; Schuldiner Shimon. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) FEBS letters, (2004 Mar 26) 562 (1-3) 193-6. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB EmrE is a multidrug **transporter** from Escherichia coli that belongs to the **Smr** family of small multidrug **transporters.** The secondary structure of EmrE consists of a four helical bundle, as judged by different techniques. EmrE has been extensively characterized; nevertheless, the membrane topology of EmrE has not been determined yet. Previous work with a homologous **Smr** protein provided partial information of the membrane topology, however the location of the carboxy-terminus remained inconclusive. In this work we probed the membrane topology of EmrE, focusing on the carboxy-terminus of the protein, using two independent approaches. Our results support a secondary structure where the carboxy-terminus faces the cytoplasm, while the first loop faces the periplasm.

L45 ANSWER 6 OF 26 MEDLINE on STN DUPLICATE 3
2003149697. PubMed ID: 12551892. Characterization of an archaeal multidrug **transporter** with a unique amino acid composition. Ninio Shira; Schuldiner Shimon. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (2003 Apr 4) 278 (14) 12000-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The **Smr** family of multidrug **transporters** consists of small membrane proteins that extrude various drugs in exchange with protons rendering cells resistant to these drugs. **Smr** proteins identified to date have been found only in Eubacteria. In this work we present the cloning and characterization of an **Smr** protein from the archaeon Halobacterium salinarum, the first **Smr** in the archaeal kingdom. The protein, named Hsmr, was identified through sequence similarity to the **Smr** family, and the DNA sequence was cloned into an Escherichia coli expression system. Hsmr is heterologously expressed in a functional form despite the difference in lipid composition of the membrane and the lower salt in the cell and its environment. Cells harboring the Hsmr plasmid transport ethidium bromide in an uncoupler-sensitive process and gain resistance to ethidium bromide and acriflavine. Hsmr binds tetraphenylphosphonium (TPP(+)) with a relatively low affinity (K(D) approximately 200 nm) at low salt concentration that increases (K(D) approximately 40 nm) upon the addition of 2 m of either NaCl or KCl. The Hsmr protein contains many of the signature sequence elements of the **Smr** family and also a high content of negative residues in the loops, characteristic of extreme halophiles. Strikingly, Hsmr is composed of over 40% valine and alanine residues. These residues are clustered at certain regions of the protein in domains that are not important for activity, as judged from lack of conservation and from previous studies with other **Smr** proteins. We suggest that this high content of alanine and valine residues is a reflection of a "natural" alanine and valine scanning necessitated by the high GC content of the gene. This phenomenon reveals significant sequence elements in small multidrug **transporters.**

L45 ANSWER 7 OF 26 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2004004662 EMBASE Efflux-mediated multiple antibiotic resistance in Pseudomonas aeruginosa. Li X.-Z.. X.-Z. Li, Verterinary Drug Directorate, Health Canada, Ottawa, Ont. K1A OK9, United States. Xianzhi_li@yahoo.com. Chinese Journal of Antibiotics 28/10 (577-596) 2003.
Refs: 134.
ISSN: 1001-8689. CODEN: KANGDS. Pub. Country: China. Language: English.
Summary Language: English; Chinese.

AB Pseudomonas aeruginosa is an opportunistic pathogen and displays high-level intrinsic and acquired multiple antimicrobial resistance. Mechanisms for such resistance are attributed by the interplay between the broad-specific multidrug efflux pumps and the low outer membrane permeability. A total of six multidrug efflux pumps, which belong to the Resistance-Nodulation-Division (RND) superfamily, have been characterized to date in P. aeruginosa. The RND efflux systems in Gram-negative bacteria are typically the three-component pumps, each composed of an inner membrane **transporter**, an inner membrane-associated periplasmic membrane fusion protein, and an outer membrane efflux channel protein. Of these known pumps of P. aeruginosa, the MexAB-OprM efflux system plays the most important role in drug resistance and shows the broadest substrate ranges that include most conventional classes of antibiotics. Regulation of these pumps is typically linked to local regulators (repressor or activators), whose mutations or inactivation produce multiple antibiotic-resistant strains as revealed in many clinical isolates. Efflux mechanisms act together with other resistance determinants to further raise resistance levels. As such, the efflux pump inhibitors are being sought to combat efflux-mediated drug resistance in order to restore antibacterial activity of antibiotics subject to efflux. Efflux reversal of fluoroquinolones in P. aeruginosa by efflux pump inhibitors has been demonstrated.

L45 ANSWER 8 OF 26 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2004:39208 Document No.: PREV200400031375. Structure and function of efflux pumps that confer resistance to drugs. Borges-Walmsley, M. Ines; McKeegan, Kenneth S.; Walmsley, Adrian R. [Reprint Author]. Centre for Infectious Diseases, Department of Biological Sciences, Wolfson Research Institute, University of Durham, Stockton Campus, Stockton-on-Tees, TS17 6BH, UK. a.r.walmsley@durham.ac.uk. Biochemical Journal, (1 December 2003) Vol. 376, No. 2, pp. 313-338. print.
ISSN: 0264-6021. Language: English.

AB Resistance to therapeutic drugs encompasses a diverse range of biological systems, which all have a human impact. From the relative simplicity of bacterial cells, fungi and protozoa to the complexity of human cancer cells, resistance has become problematic. Stated in its simplest terms, drug resistance decreases the chance of providing successful treatment against a plethora of diseases. Worryingly, it is a problem that is increasing, and consequently there is a pressing need to develop new and effective classes of drugs. This has provided a powerful stimulus in promoting research on drug resistance and, ultimately, it is hoped that this research will provide novel approaches that will allow the deliberate circumvention of well understood resistance mechanisms. A major mechanism of resistance in both microbes and cancer cells is the membrane protein-catalysed extrusion of drugs from the cell. Resistant cells exploit proton-driven anti-porters and/or ATP-driven ABC (ATP-binding cassette) **transporters** to extrude cytotoxic drugs that usually enter the cell by passive diffusion. Although some of these drug efflux pumps transport specific substrates, many are **transporters** of multiple substrates. These multidrug pumps can often transport a variety of structurally unrelated hydrophobic compounds, ranging from dyes to lipids. If we are to nullify the effects of efflux-mediated drug resistance, we must first of all understand how these efflux pumps can accommodate a diverse range of compounds and, secondly, how conformational changes in these proteins are coupled to substrate translocation. These are key questions that must be addressed. In this review we report on the advances that have been made in understanding the structure and function of drug efflux pumps.

L45 ANSWER 9 OF 26 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:417320 Document No.: PREV200300417320. Cell-free expression of membrane proteins: Preparative scale production and labeling of multidrug **transporters.** Klammt, C. [Reprint Author]; Rueterjans, H. [Reprint Author]; Bernhard, F. [Reprint Author]; Ziehm, T. [Reprint Author]; Lorch, M. [Reprint Author]; Glaubitz, C. [Reprint Author]. Inst. of Biophysical Chemistry, University of Frankfurt, Frankfurt, Germany. European Biophysics Journal, (June 2003) Vol. 32, No. 3, pp. 220. print.
Meeting Info.: 4th European Biophysics Congress. Alicante, Spain. July 05-09, 2003. European Biophysical Societies' Association (EBSA); Spanish Biophysics Society.
CODEN: EBJOE8. ISSN: 0175-7571. Language: English.

L45 ANSWER 10 OF 26 MEDLINE on STN DUPLICATE 4

2003366220. PubMed ID: 12900034. Characterization of In111, a class 1 integron that carries the extended-spectrum beta-lactamase gene blaIBC-1. Vourli S; Tzouvelekis L S; Tzelepi E; Lebessi E; Legakis N J; Miriagou V. (Laboratory of Bacteriology, Hellenic Pasteur Institute, Vas. Sofias 127, Athens 11521, Greece.) FEMS microbiology letters, (2003 Aug 8) 225 (1) 149-53. Journal code: 7705721. ISSN: 0378-1097. Pub. country:

Netherlands. Language: English.

AB A class 1 integron, In111, carried by a self-transferable plasmid from an Escherichia coli clinical strain was characterized. The variable region of In111 constituted an array of gene cassettes encoding the extended-spectrum beta-lactamase IBC-1, the aminoglycoside-modifying enzymes AAC(6')-Ib and ANT(3")-Ia, dihydrofolate reductase I and a putative polypeptide (**SMR**-2) sharing similarity with the Qac **transporters.** Transcription of the gene cassettes was driven by a hybrid-type P1 promoter located in a typical 5' conserved segment (CS). The 3'CS included sulI, qacEDelta1, orf5 and orf6. In111 was bounded on the right by an inversely oriented IRt. The 5'CS was preceded by an intact IS26 element followed by an aphA1 gene.

L45 ANSWER 11 OF 26 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:601714 The Genuine Article (R) Number: 698CP. Examination of EmrE conformational differences in various membrane mimetic environments. Federkell S L; Winstone T L; Jickling G; Turner R J (Reprint). Univ Calgary, Dept Biol Sci, Div Biochem, 2500 Univ Dr NW, Calgary, AB T2N 1N4, Canada (Reprint); Univ Calgary, Dept Biol Sci, Div Biochem, Calgary, AB T2N 1N4, Canada. BIOCHEMISTRY AND CELL BIOLOGY-BIOCHIMIE ET BIOLOGIE CELLULAIRE (APR 2003) Vol. 81, No. 2, pp. 61-70. Publisher: NATL RESEARCH COUNCIL CANADA. RESEARCH JOURNALS, MONTREAL RD, OTTAWA, ONTARIO K1A 0R6, CANADA. ISSN: 0829-8211. Pub. country: Canada. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Ethidium multidrug resistance protein (EmrE) is a member of the small multidrug resistance family of proteins and is responsible for resistance in Escherichia coli to a diverse group of lipophilic cations. Research is beginning to elucidate structural information as well as substrate binding and extrusion mechanisms for this protein. However, the choice of membrane mimetic environment to perform structural studies needs to be made. In this study EmrE was solubilized in different membrane mimetic environments to investigate the influence of environment on the structure and dynamics of the protein by comparing the fluorescence properties of emission maxima, peak shifts, relative intensities, acrylamide quenching constants, and polarization. Taken together, the different fluorescence observations on EmrE in the various membrane mimetic systems tested suggest that the tryptophan residues in EmrE are present in the most flexible and exposed state when solubilized in methanol, followed by sodium dodecyl sulfate and urea. The two detergents N-dodecyl-beta-D-maltoside (DM) and polyoxyethylene(8)dodecyl ether, for the most part, only display subtle differences between the spectral properties with DM best representing the lipid environment. The conformation of EmrE is clearly more open and dynamic in detergent relative to being reconstituted in small unilamellar vesicles. The fluorescence observations of EmrE solubilized in trifluoroethanol shows an environment that is similar to that of EmrE solubilized in detergents. Additionally, secondary structure was monitored by circular dichroism (CD). The CD spectra were similar among the different solubilizing conditions, suggesting little difference in a-helical content. This work establishes groundwork for the choice of solubilizing conditions for future structural, folding, and ligand binding studies.

L45 ANSWER 12 OF 26 CAPLUS COPYRIGHT 2005 ACS on STN

2005:32451 Genomic diversity of antiseptic resistance genes in staphylococci. Kobayashi, Nobumichi; Alam, Mohammed Mahbub; Ishino, Masaho (Department of Hygiene, Sapporo Medical University School of Medicine, Chuo-ku, Sapporo, 060-8556, Japan). Recent Research Developments in Antimicrobial Agents & Chemotherapy, 6, 53-64 (English) 2003. CODEN: RDACFH. Publisher: Research Signpost.

AB Antiseptic resistance of staphylococci is caused by drug efflux pump (**transporter**) proteins located on bacterial membrane. There are two distinct groups of antiseptic resistance proteins represented by QacA/QacB and QacC/**Smr**, which are associated with high-level and low-level antiseptic resistance in staphylococci, resp. Structurally, the

QacA/QacB and QacC/**Smr** belong to the major facilitator superfamily (MFS) and small multidrug resistance (**SMR**) family, resp. **SMR** family includes various antiseptic resistance proteins which have been found in staphylococci as well as in other bacterial species. Phylogenetic anal. of the **SMR** family proteins indicated the presence of three genetic clusters (genogroup 1 .apprx. 3) suggesting that genetic divergence among genes of **SMR** family might have occurred sep. in Gram-pos. bacteria and Gram-neg. bacteria. Through anal. of gene cassette of **smr** in Staphylococcus aureus, evolutionary original form (Type 1 cassette) and genetically modified forms (Type 2 and Type 3 cassette, and other variations) were found, indicating occurrence of frequent genomic modification in cassette structure of this gene. The QacA/QacB proteins found in staphylococci are genetically closely related each other and sequence diversity is extremely low. However, QacA and QacB are different in their antiseptic resistance phenotypes. Therefore, direction of genomic divergence between qacA gene group and qacB gene group has been an important issue with respect to the relationship between genetic evolution and change in resistance level. In our study on genomic diversity in qacA/B and its downstream region, more divergent forms of these genomic portions were found in qacB than in qacA, suggesting that qacA might be an evolutionary origin and most qacB might have been derived from qacA. Genetic evolution of resistance genes, which may sometimes accompany phenotypic change, should be surveyed constantly for control of resistant bacteria.

L45 ANSWER 13 OF 26 MEDLINE on STN DUPLICATE 5

2002736582. PubMed ID: 12499164. Contributions of MexAB-OprM and an EmrE homolog to intrinsic resistance of Pseudomonas aeruginosa to aminoglycosides and dyes. Li Xian-Zhi; Poole Keith; Nikaido Hiroshi. (Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3206, USA.) Antimicrobial agents and chemotherapy, (2003 Jan) 47 (1) 27-33. Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB Of the six putative small multidrug resistance (**SMR**) family proteins of Pseudomonas aeruginosa, a protein encoded by the PA4990 gene (emrE(Pae)) shows the highest identity to the well-characterized EmrE efflux **transporter** of Escherichia coli. Reverse transcription-PCR confirmed the expression of emrE(Pae) in the wild-type strain of P. aeruginosa. Using isogenic emrE(Pae), mexAB-oprM, and/or mexB deletion mutants, the contributions of the EmrE protein and the MexAB-OprM efflux system to drug resistance in P. aeruginosa were assessed by a drug susceptibility test carried out in a low-ionic-strength medium, Difco nutrient broth. We found that EmrE(Pae) contributed to intrinsic resistance not only to ethidium bromide and acriflavine but also to aminoglycosides. In this low-ionic-strength medium, MexAB-OprM was also shown to contribute to aminoglycoside resistance, presumably via active efflux. Aminoglycoside resistance caused by these two pumps could not be demonstrated in high-ionic-strength media, such as Luria broth or Mueller-Hinton broth. The EmrE-dependent efflux of ethidium bromide was confirmed by a continuous fluorescence assay.

L45 ANSWER 14 OF 26 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:278286 Document No.: PREV200200278286. Precious things come in little packages. Schuldiner, Shimon [Reprint author]; Granot, Dorit; Steiner Mordoch, Sonia; Ninio, Shira; Rotem, Dvir; Soskin, Michael; Yerushalmi, Hagit. Alexander Silberman Inst. Life Sciences, Hebrew University of Jerusalem, 91904, Jerusalem, Israel. shimon.schuldiner@mail.ls.huji.ac.il. Paulsen, Ian T. [Editor]; Lewis, Kim [Editor]. (2002) pp. 31-48. JMMB Symposium Series. Microbial multidrug efflux. print. Publisher: Horizon Scientific Press, 32 Hewitts Lane, Wymondham, Norfolk, NR18 0JA, UK. Series: JMMB Symposium Series.
ISBN: 1-898486-33-6 (cloth). Language: English.

L45 ANSWER 15 OF 26 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:584792 Document No.: PREV200200584792. Functional comparison of the small multidrug resistance proteins, Sug and EmrE, through directed evolution. Son, M. S. [Reprint author]; Del Castilho, C. [Reprint author]; Turner, R. J. [Reprint author]. University of Calgary, Calgary, AB, Canada. Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 26. print.

Meeting Info.: 102nd General Meeting of the American Society for Microbiology. Salt Lake City, UT, USA. May 19-23, 2002. American Society for Microbiology.

ISSN: 1060-2011. Language: English.

AB Sug and EmrE are both small multidrug resistance (**SMR**) proteins. EmrE is an example of the subclass that is known to actively transport quaternary ammonium compounds (QAC) which gives rise to resistance. Sug does not confer resistance to these compounds nor is transport demonstrated. Key sequence motifs are conserved across the **SMR** family of proteins. Additionally, there are key residues conserved within each sub group but not between Sugs and QAC **transporters.** In this study we have used a directed evolution approach to see what is necessary to convert a Sug to a QAC **transporter.** Four key residues, (H24, M39, I43, A44) conserved within Sug, but conserved differently within the QAC **transporters**, were chosen to convert Sug to an EmrE. Investigations were conducted into the resistance profiles of these Sug mutants and combinations thereof, as compared to wild type Sug and EmrE. Altogether, seven mutants were generated, and minimal inhibitory concentration growth curves were determined in the presence of representative QAC compounds (examples: ethidium bromide, methyltriphenylphosphonium, proflavin and methyl viologen). Surprisingly, the mutated Sugs demonstrated an increased sensitivity to the different QACs as compared to the wild type Sug and EmrE. The seven mutants, as well as wild type Sug and EmrE, which served as controls, were FLAG epitope tagged at the C-terminal end. The FLAG tag allowed for the relative quantitation of the wild type and mutant proteins, through Western blotting. The relative levels of protein accumulation in the membrane were found to be equal for all mutants. Additionally, orientation studies were conducted using maltose-binding protein as a topological reporter. The results show that the topology of Sug and EmrE has their N-terminal in the cytoplasm. The results imply that Sug can be converted to a QAC **transporter** with only a single mutation. However, because hypersensitivity was observed this protein may be an importer.

L45 ANSWER 16 OF 26 MEDLINE on STN DUPLICATE 6

2001698260. PubMed ID: 11574548. Functional analysis of novel multidrug **transporters** from human pathogens. Ninio S; Rotem D; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (2001 Dec 21) 276 (51) 48250-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Proteins of the **Smr** family are the smallest multidrug **transporters**, about 110 amino acids long, that extrude various drugs in exchange with protons, thereby rendering bacteria resistant to these compounds. One of these proteins, EmrE, is an Escherichia coli protein, which has been cloned based on its ability to confer resistance to ethidium and methyl viologen and which has been extensively characterized. More than 60 genes coding for **Smr** proteins have been identified in several bacteria based on amino acid sequence similarity to the emrE gene. In this work we have analyzed the sequence similarity among these homologues and identified some distinct signature sequence elements and several fully conserved residues. Five of these homologues, from human pathogens Mycobacterium tuberculosis, Bordetella pertussis, and Pseudomonas aeruginosa and from Escherichia coli, were cloned into an E. coli expression system. The proteins were further characterized and show varying degrees of methyl viologen uptake into proteoliposomes and [(3)H]TPP binding in solubilized membranes. The homologues can also form mixed oligomers with EmrE that exhibit intermediate binding characteristics. A comparative study of various homologous proteins provides a tool for deciphering structure-function relationship and monomer-monomer interaction in multidrug **transporters** and in membrane proteins in general.

L45 ANSWER 17 OF 26 MEDLINE on STN DUPLICATE 7

2001376573. PubMed ID: 11432728. The drug/metabolite **transporter** superfamily. Jack D L; Yang N M; Saier M H Jr. (Department of Biology, University of California at San Diego, La Jolla, CA 92093-0116, USA.) European journal of biochemistry / FEBS, (2001 Jul) 268 (13) 3620-39. Ref: 83. Journal code: 0107600. ISSN: 0014-2956. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Previous work defined several families of secondary active **transporters**, including the prokaryotic small multidrug resistance (**SMR**) and rhamnose **transporter** (RhaT) families as well as the eukaryotic organellar triose phosphate **transporter** (TPT) and nucleotide-sugar **transporter** (NST) families. We show that these families as well as several other previously unrecognized families of established or putative secondary active **transporters** comprise a large ubiquitous superfamily found in bacteria, archaea and eukaryotes. We have designated it the drug/metabolite **transporter** (DMT) superfamily (**transporter** classification number 2.A.7) and have shown that it consists of 14 phylogenetic families, five of which include no functionally well-characterized members. The largest family in the DMT superfamily, the drug/metabolite exporter (DME) family, consists of over 100 sequenced members, several of which have been implicated in metabolite export. Each DMT family consists of proteins with a distinctive topology: four, five, nine or 10 putative transmembrane alpha helical spanners (TMSs) per polypeptide chain. The five TMS proteins include an N-terminal TMS lacking the four TMS proteins. The full-length proteins of 10 putative TMSs apparently arose by intragenic duplication of an element encoding a primordial five-TMS polypeptide. Sequenced members of the 14 families are tabulated and phylogenetic trees for all the families are presented. Sequence and topological analyses allow structural and functional predictions.

L45 ANSWER 18 OF 26 MEDLINE on STN DUPLICATE 8

2001293981. PubMed ID: 11378963. **SMR**-type multidrug resistance pumps. Chung Y J; Saier M H Jr. (Department of Biology, University of California at San Diego, La Jolla, CA 92093, USA.) Current opinion in drug discovery & development, (2001 Mar) 4 (2) 237-45. Ref: 29. Journal code: 100887519. ISSN: 1367-6733. Pub. country: England: United Kingdom. Language: English.

AB Multidrug resistance (MDR) efflux pumps in pathogenic microorganisms nullify the effects of antimicrobial drugs used in medicine. We have conducted phylogenetic analyses showing that these efflux pumps are associated with five superfamilies of transport systems. One of these, the drug/metabolite **transporter** (DMT) superfamily includes a family of small multidrug resistance (**SMR**)-conferring proteins that are discussed in detail in this review. A single microorganism such as Bacillus subtilis may possess multiple homologs of this family, and these homologs are believed to form both homo-oligomeric or hetero-oligomeric pumps, some of which export cationic drugs. The characteristics of some of these systems and the genes that encode them are described, with emphasis on the eight homologs encoded within the B subtilis genome. Anomalies and unanswered questions that provide impetus for future studies are presented.

L45 ANSWER 19 OF 26 MEDLINE on STN DUPLICATE 9

2001483519. PubMed ID: 11321568. Precious things come in little packages. Schuldiner S; Granot D; Steiner S; Ninio S; Rotem D; Soskin M; Yerushalmi H. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Israel.) Journal of molecular microbiology and biotechnology, (2001 Apr) 3 (2) 155-62. Ref: 55. Journal code: 100892561. ISSN: 1464-1801. Pub. country: England: United Kingdom. Language: English.

AB The 110-amino acid multidrug **transporter** from E. coli, EmrE, is a member of the family of MiniTexan or **Smr** drug **transporters.** EmrE can transport acriflavine, ethidium bromide, tetraphenylphosphonium (TPP+), benzalkonium and several other drugs with relatively high affinities. EmrE is an H+/drug antiporter, utilizing the proton electrochemical gradient generated across the bacterial cytoplasmic membrane by exchanging two protons with one substrate molecule. The EmrE multidrug **transporter** is unique in its small size and hydrophobic nature. Hydropathic analysis of the EmrE sequence predicts four alpha-helical transmembrane segments. This model is experimentally supported by FTIR studies that confirm the high alpha-helicity of the protein and by high-resolution heteronuclear NMR analysis of the protein structure. The TMS of EmrE are tightly packed in the membrane without any continuous aqueous domain, as was shown by Cysteine scanning experiments. These results suggest the existence of a hydrophobic pathway through which the substrates are translocated. EmrE is functional as a homo-oligomer as suggested by several lines of evidence, including co-reconstitution experiments of wild-type protein with inactive mutants in which negative dominance has been observed. EmrE has only one membrane embedded charged residue, Glu-14, that is conserved in more than fifty homologous proteins and it is a simple model system to study the role of carboxylic residues in ion-coupled **transporters.** We have used mutagenesis and chemical modification to show that Glu-14 is part of the substrate-binding site. Its role in proton binding and translocation was shown by a study of the effect of pH on ligand binding, uptake, efflux and exchange reactions. We conclude that Glu-14 is an essential part of a binding site, common to substrates and protons. The occupancy of this site is mutually exclusive and provides the basis of the simplest coupling of two fluxes. Because of some of its properties and its size, EmrE provides a unique system to understand mechanisms of substrate recognition and translocation.

L45 ANSWER 20 OF 26 CAPLUS COPYRIGHT 2005 ACS on STN

2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L45 ANSWER 21 OF 26 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2000:268021 The Genuine Article (R) Number: 299NU. A broad-specificity multidrug efflux pump requiring a pair of homologous **SMR**-type proteins. Jack D L; Storms M L; Tchieu J H; Paulsen I T; Saier M H (Reprint). UNIV CALIF SAN DIEGO, DEPT BIOL, LA JOLLA, CA 92093 (Reprint); UNIV CALIF SAN DIEGO, DEPT BIOL, LA JOLLA, CA 92093. JOURNAL OF BACTERIOLOGY (APR 2000) Vol. 182, No. 8, pp. 2311-2313. Publisher: AMER SOC MICROBIOLOGY. 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171 . ISSN: 0021-9193. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The Bacillus subtilis genome encodes seven homologues of the small multidrug resistance (**SMR**) family of drug efflux pumps. Six of these homologues are paired in three distinct operons, and coexpression in Escherichia coli of one such operon, ykkCD, but not expression of either ykkC or ykkD alone, gives rise to a broad specificity, multidrug-resistant phenotype including resistance to cationic, anionic, and neutral drugs.

L45 ANSWER 22 OF 26 MEDLINE on STN DUPLICATE 10

2000408812. PubMed ID: 10874120. Antibiotic efflux pumps. Van Bambeke F; Balzi E; Tulkens P M. (Unite de Pharmacologie Cellulaire et Moleculaire, Universite Catholique de Louvain, Brussels, Belgium.. vanbambeke@facm.ucl.ac.be) . Biochemical pharmacology, (2000 Aug 15) 60 (4) 457-70. Ref: 118. Journal code: 0101032. ISSN: 0006-2952. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Active efflux from procaryotic as well as eucaryotic cells strongly modulates the activity of a large number of antibiotics. Effective antibiotic transport has now been observed for many classes of drug efflux pumps. Thus, within the group of primary active **transporters**, predominant in eucaryotes, six families belonging to the ATP-binding cassette superfamily, and including the P-glycoprotein in the MDR (Multi Drug Resistance) group and the MRP (Multidrug Resistance Protein), have been recognized as being responsible for antibiotic efflux. Within the class of secondary active **transporters** (antiports, symports, and uniports), ten families of antibiotic efflux pumps have been described, distributed in five superfamilies [**SMR** (Small Multidrug Resistance), MET (Multidrug Endosomal **Transporter**), MAR (Multi Antimicrobial Resistance), RND (Resistance Nodulation Division), and MFS (Major Facilitator Superfamily)]. Nowadays antibiotic efflux pumps are believed to contribute significantly to acquired bacterial resistance because of the very broad variety of substrates they recognize, their expression in important pathogens, and their cooperation with other mechanisms of resistance. Their presence also explains high-level intrinsic resistances found in specific organisms. Stable mutations in regulatory genes can produce phenotypes of irreversible multidrug resistance. In eucaryotes, antibiotic efflux pumps modulate the accumulation of antimicrobials in phagocytic cells and play major roles in their transepithelial transport. The existence of antibiotic efflux pumps, and their impact on therapy, must now be taken fully into account for the selection of novel antimicrobials. The design of specific, potent inhibitors appears to be an important goal for the improved control of infectious diseases in the near future.

L45 ANSWER 23 OF 26 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:4259 Document No.: PREV200100004259. Staphylococcus aureus (SA) resistance to thrombin-induced platelet microbicidal protein (tPMP-1) mediated by the qacA gene is specific and independent of multidrug efflux pump activity. Kupferwasser, L. I. [Reprint author]; Skurray, R. A.; Firth, N.; Brown, M. H.; Yeaman, M. R. [Reprint author]; Prasad, R.; Smriti, M.; Bayer, A. S. [Reprint author]. Harbor-UCLA Med. Ctr., Torrance, CA, USA. Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2000) Vol. 40, pp. 133. print. Meeting Info.: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. Toronto, Ontario, Canada. September 17-20, 2000. Interscience Conference on Antimicrobial Agents and Chemotherapy; American Society of Microbiology.
Language: English.

L45 ANSWER 24 OF 26 MEDLINE on STN DUPLICATE 11

1999280180. PubMed ID: 10353713. Alpha-periodicity analysis of small multidrug resistance (**SMR**) efflux **transporters**.
Edwards R A; Turner R J. (Department of Biological Sciences, University of Calgary, AB, Canada.) Biochemistry and cell biology = Biochimie et biologie cellulaire, (1998) 76 (5) 791-7. Journal code: 8606068. ISSN: 0829-8211. Pub. country: Canada. Language: English.

AB Proteins in the small multidrug resistance (**SMR**) family of transport proteins are about 110 amino acids in length and are predicted to have four transmembrane helices. This family is divided into a two groups, one of which we have referred to as small multidrug pumps (Smp) and confer resistance to a wide variety of quaternary ammonium compounds through a proton-drug efflux antiport mechanism. Members of the second group within this family have, as yet, not had their substrate profile characterized and are referred to as Sug proteins. Alpha-periodicity analysis was conducted on a set of six homologous proteins of the **SMR** family consisting of three established Smp and three Sug proteins. Several amino acid properties were used in the analysis including hydropathy, variability, and a substitution matrix for lipid exposed amino acids. The scanning window was varied between 8 and 14 residues and the alpha-periodicity was calculated from the peaks in the Fourier transform power spectra in the region between 3.0 and 4.3 residues/turn. This analysis adds to the hydropathy analysis to give a more confident prediction of which residues are within the lipid bilayer for each of the four transmembrane helices. Information was also obtained that allowed for the identification of zones within each transmembrane helix that face the interior of the helical bundle on one side and are lipid exposed on the other face.

L45 ANSWER 25 OF 26 CAPLUS COPYRIGHT 2005 ACS on STN
1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US9491 19950801. PRIORITY: US 1994-286430 19940805.

AB Polyspecific immunoconjugates and antibody composites that bind a multidrug **transporter** protein and an antigen associated with a tumor or infectious agent are used to overcom the multidrug resistant phenotype. These immunoconjugates and composites also can be used diagnostically to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents. In example, polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen bispecific antibody were prepared for colon cancer treatment. 99mTc- and 111Indium-labeled polyspecific immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa were also prepared

L45 ANSWER 26 OF 26 MEDLINE on STN DUPLICATE 12
97140959. PubMed ID: 8987357. Proton-dependent multidrug efflux systems. Paulsen I T; Brown M H; Skurray R A. (School of Biological Sciences, University of Sydney, New South Wales, Australia.) Microbiological reviews, (1996 Dec) 60 (4) 575-608. Ref: 326. Journal code: 7806086. ISSN: 0146-0749. Pub. country: United States. Language: English.

AB Multidrug efflux systems display the ability to transport a variety of structurally unrelated drugs from a cell and consequently are capable of conferring resistance to a diverse range of chemotherapeutic agents. This review examines multidrug efflux systems which use the proton motive force to drive drug transport. These proteins are likely to operate as multidrug/proton antiporters and have been identified in both prokaryotes and eukaryotes. Such proton-dependent multidrug efflux proteins belong to three distinct families or superfamilies of transport proteins: the major facilitator superfamily (MFS), the small multidrug resistance (**SMR**) family, and the resistance/ nodulation/cell division (RND) family. The MFS consists of symporters, antiporters, and uniporters with either 12 or 14 transmembrane-spanning segments (TMS), and we show that within the MFS, three separate families include various multidrug/proton antiport

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          proteins.  The SMR family consists of proteins with four TMS,
          and the multidrug efflux proteins within this family are the smallest
          known secondary transporters.  The RND family consists of 12-TMS
          transport proteins and includes a number of multidrug efflux proteins with
          particularly broad substrate specificity.  In gram-negative bacteria, some
          multidrug efflux systems require two auxiliary constituents, which might
          enable drug transport to occur across both membranes of the cell envelope.
          These auxiliary constituents belong to the membrane fusion protein and the
          outer membrane factor families, respectively.  This review examines in
          detail each of the characterized proton-linked multidrug efflux systems.
          The molecular basis of the broad substrate specificity of these
          transporters is discussed.  The surprisingly wide distribution of
          multidrug efflux systems and their multiplicity in single organisms, with
          Escherichia coli, for instance, possessing at least nine proton-dependent
          multidrug efflux systems with overlapping specificities, is examined.  We
          also discuss whether the normal physiological role of the multidrug efflux
          systems is to protect the cell from toxic compounds or whether they fulfil
          primary functions unrelated to drug resistance and only efflux multiple
          drugs fortuitously or opportunistically.


=> s l11 and "QacE"
L46           6 L11 AND "QACE"

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L47            4 DUP REMOVE L46 (2 DUPLICATES REMOVED)

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L47  ANSWER 1 OF 4  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN                                                  DUPLICATE 1
2004502644  EMBASE  Antibiotic multiresistance plasmid pRSB101 isolated from a
     wastewater treatment plant is related to plasmids residing in
     phytopathogenic bacteria and carries eight different resistance
     determinants including a multidrug transport system. Szczepanowski R.;
     Krahn I.; Linke B.; Goesmann A.; Puhler A.; Schluter A.. A. Schluter,
     Fakultat fur Biologie, Lehrstuhl fur Genetik, Universitat Bielefeld,
     Postfach 100131, D-33501 Bielefeld, Germany. andreas.schlueter@genetik.uni-
     bielefeld.de. Microbiology  150/11 (3613-3630)  2004.
     Refs: 89.
     ISSN: 1350-0872. CODEN: MROBEO. Pub. Country: United Kingdom. Language:
     English. Summary Language: English.
AB   Ten different antibiotic resistance plasmids conferring high-level
     erythromycin resistance were isolated from an activated sludge bacterial
     community of a wastewater treatment plant by applying a
     transformation-based approach. One of these plasmids, designated pRSB101,
     mediates resistance to tetracycline, erythromycin, roxythromycin,
     sulfonamides, cephalosporins, spectinomycin, streptomycin, trimethoprim,
     nalidixic acid and low concentrations of norfloxacin. Plasmid pRSB101 was
     completely sequenced and annotated. Its size is 47 829 bp. Conserved
     synteny exists between the pRSB101 replication/partition (rep/par) module
     and the pXAC33-replicon from the phytopathogen Xanthomonas axonopodis pv.
     citri. The second pRSB101 backbone module encodes a three-Mob-protein type
     mobilization (mob) system with homology to that of IncQ-like plasmids.
     Plasmid pRSB101 is mobilizable with the help of the IncP-1α plasmid
     RN4 providing transfer functions in trans. A 20 kb resistance region on
     pRSB101 is located within an integron-containing Tn402-like transposon.
     The variable region of the class 1 integron carries the genes dhfr1 for a
     dihydrofolate reductase, aadA2 for a spectinomycin/streptomycin
     adenylyltransferase and bla(TLA-2) for a so far unknown Ambler class A
     extended spectrum β-lactamase. The integron-specific 3'-segment (
     qacE.DELTA.1-sul1-orf5Δ) is connected to a macrolide
     resistance operon consisting of the genes mph(A) (macrolide
     2'-phosphotransferase I), mrx (hydrophobic protein of unknown function)
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and mphR(A) (regulatory protein). Finally, a putative mobile element with the tetracycline resistance genes tetA (tetracycline efflux pump) and tetR was identified upstream of the Tn402-specific transposase gene tniA. The second 'genetic load' region on pRSB101 harbours four distinct mobile genetic elements, another integron belonging to a new class and footprints of two more transposable elements. A tripartite multidrug (MDR) **transporter** consisting of an ATP-binding-cassette (ABC)-type ATPase and permease, and an efflux membrane fusion protein (MFP) of the RND-family is encoded between the replication/partition and the mobilization module. Homologues of the macrolide resistance genes mph(A), mrx and mphR(A) were detected on eight other erythromycin resistance-plasmids isolated from activated sludge bacteria. Plasmid pRSB101-like repA amplicons were also obtained from plasmid-DNA preparations of the final effluents of the wastewater treatment plant indicating that pRSB101-like plasmids are released with the final effluents into the environment. .COPYRGT. 2004 SGM.

L47 ANSWER 2 OF 4 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 2
2003424081 EMBASE Characterization of In111, a class 1 integron that carries the extended-spectrum β-lactamase gene bla(IBC-1). Vourli S.; Tzouvelekis L.S.; Tzelepi E.; Lebessi E.; Legakis N.J.; Miriagou V.. V. Miriagou, Laboratory of Bacteriology, Hellenic Pasteur Institute, Vas. Sofias 127, Athens 11521, Greece. miriagou@otenet.gr. FEMS Microbiology Letters 225/1 (149-153) 8 Aug 2003.
Refs: 27.
ISSN: 0378-1097. CODEN: FMLED7. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB A class 1 integron, In111, carried by a self-transferable plasmid from an Escherichia coli clinical strain was characterized. The variable region of In111 constituted an array of gene cassettes encoding the extended-spectrum β-lactamase IBC-1, the aminoglycoside-modifying enzymes AAC(6')-Ib and ANT(3")-Ia, dihydrofolate reductase I and a putative polypeptide (SMR-2) sharing similarity with the Qac **transporters.** Transcription of the gene cassettes was driven by a hybrid-type P1 promoter located in a typical 5' conserved segment (CS). The 3'CS included sulI, **qacE**.DELTA.1, orf5 and orf6. In111 was bounded on the right by an inversely oriented IRt. The 5'CS was preceded by an intact IS26 element followed by an aphA1 gene. .COPYRGT. 2003 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

L47 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L47 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype.

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          Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
          A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
          CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
          KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
          SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
          DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
          TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
          PRIORITY: US 1994-286430 19940805.
AB        Polyspecific immunoconjugates and antibody composites that bind a
          multidrug transporter protein and an antigen associated with a
          tumor or infectious agent are used to overcom the multidrug resistant
          phenotype.  These immunoconjugates and composites also can be used
          diagnostically to determine whether the failure of traditional chemotherapy is
          due to the presence of multidrug resistant tumor cells, multidrug
          resistant HIV-infected cells or multidrug resistant infectious agents.  In
          example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
          doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
          bispecific antibody were prepared for colon cancer treatment.  99mTc- and
          111Indium-labeled polyspecific immunoconjugate targeted to multidrug
          resistant Pseudomonas aeruginosa were also prepared


=> s l11 and "MvrC"
L48          42 L11 AND "MVRC"

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L49          38 DUP REMOVE L48 (4 DUPLICATES REMOVED)

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L49  ANSWER 1 OF 38     MEDLINE on STN
2004569820.     PubMed ID: 15371426.    EmrE, a multidrug transporter
          from Escherichia coli, transports monovalent and divalent substrates with
          the same stoichiometry. Rotem Dvir; Schuldiner Shimon. (Alexander
          Silberman Institute of Life Sciences, Hebrew University of Jerusalem,
          91904 Jerusalem, Israel. ) Journal of biological chemistry, (2004 Nov 19)
          279 (47) 48787-93.  Journal code: 2985121R. ISSN: 0021-9258. Pub. country:
          United States. Language: English.
AB        Multidrug transporters recognize and transport substrates with
          apparently little common structural features.  At times these substrates
          are neutral, negatively, or positively charged, and only limited
          information is available as to how these proteins deal with the energetic
          consequences of transport of substrates with different charges.  Multidrug
          transporters and drug-specific efflux systems are responsible for
          clinically significant resistance to chemotherapeutic agents in pathogenic
          bacteria, fungi, parasites, and human cancer cells.  Understanding how
          these efflux systems handle different substrates may also have practical
          implications in the development of strategies to overcome the resistance
          mechanisms mediated by these proteins.  Here, we compare transport of
          monovalent and divalent substrates by EmrE, a multidrug
          transporter from Escherichia coli, in intact cells and in
          proteoliposomes reconstituted with the purified protein.  The results
          demonstrated that whereas the transport of monovalent substrates involves
          charge movement (i.e. electrogenic), the transport of divalent substrate
          does not (i.e. electroneutral).  Together with previous results, these
          findings suggest that an EmrE dimer exchanges two protons per substrate
          molecule during each transport cycle.  In intact cells, under conditions
          where the only driving force is the electrical potential, EmrE confers
          resistance to monovalent substrates but not to divalent ones.  In the
          presence of proton gradients, resistance to both types of substrates is
          detected.  The finding that under some conditions EmrE does not remove
          certain types of drugs points out the importance of an in-depth
          understanding of mechanisms of action of multidrug transporters
          to devise strategies for coping with the problem of multidrug resistance.
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L49 ANSWER 2 OF 38 MEDLINE on STN

2004114036. PubMed ID: 14701800. Direct evidence for substrate-induced proton release in detergent-solubilized EmrE, a multidrug **transporter.** Soskine Misha; Adam Yoav; Schuldiner Shimon. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Israel.) Journal of biological chemistry, (2004 Mar 12) 279 (11) 9951-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A novel approach to study coupling of substrate and ion fluxes is presented. EmrE is an H(+)-coupled multidrug **transporter** from Escherichia coli. Detergent-solubilized EmrE binds substrate with high affinity in a pH-dependent mode. Here we show, for the first time in an ion-coupled **transporter**, substrate-induced release of protons in a detergent-solubilized preparation. The direct measurements allow for an important quantitation of the phenomenon. Thus, stoichiometry of the release in the wild type and a mutant with a single carboxyl at position 14 is very similar and about 0.8 protons/monomer. The findings demonstrate that the only residue involved in proton release is a highly conserved membrane-embedded glutamate (Glu-14) and that all the Glu-14 residues in the EmrE functional oligomer participate in proton release. Furthermore, from the pH dependence of the release we determined the pK of Glu-14 as 8.5 and for an aspartate replacement at the same position as 6.7. The high pK of the carboxyl at position 14 is essential for coupling of fluxes of protons and substrates.

L49 ANSWER 3 OF 38 MEDLINE on STN

2004106930. PubMed ID: 14970332. Structure of the multidrug resistance efflux **transporter** EmrE from Escherichia coli. Ma Che; Chang Geoffrey. (Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, CB-105, La Jolla, CA 92037.) Proceedings of the National Academy of Sciences of the United States of America, (2004 Mar 2) 101 (9) 2852-7. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Multidrug resistance efflux **transporters** threaten to reverse the progress treating infectious disease by extruding a wide range of drug and other cytotoxic compounds. One such drug **transporter**, EmrE, from the small multidrug resistance family, utilizes proton gradients as an energy source to drive substrate translocation. In an effort to understand the molecular structural basis of this transport mechanism, we have determined the structure of EmrE from Escherichia coli to 3.8 A. EmrE is a tetramer comprised of two conformational heterodimers related by a pseudo two-fold symmetry axis perpendicular to the cell membrane. Based on the structure and biochemical evidence, we propose a mechanism by which EmrE accomplishes multidrug efflux by coupling conformational changes between two heterodimers with proton gradient. Because of its simplicity and compact size, the structure of EmrE can serve as an ideal model for understanding the general structural basis of proton:drug antiport for other drug efflux systems.

L49 ANSWER 4 OF 38 MEDLINE on STN

2004070634. PubMed ID: 14755055. In vitro synthesis of fully functional EmrE, a multidrug **transporter**, and study of its oligomeric state. Elbaz Yael; Steiner-Mordoch Sonia; Danieli Tsafi; Schuldiner Shimon. (Protein Expression Facility, Wolfson Foundation Center for Applied Structural Biology, Hebrew University of Jerusalem, Jerusalem 91904, Israel.) Proceedings of the National Academy of Sciences of the United States of America, (2004 Feb 10) 101 (6) 1519-24. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB EmrE is a small multidrug **transporter** from Escherichia coli that provides a unique model for the study of polytopic membrane proteins. Here, we show its synthesis in a cell-free system in a fully functional form. The detergent-solubilized protein binds substrates with high affinity and, when reconstituted into proteoliposomes, transports substrate in a Deltamicro(H)(+)-dependent fashion. Here, we used the cell-free system to study the oligomeric properties of EmrE. EmrE functions as an oligomer, but the size of the functional oligomer has not been established unequivocally. Coexpression of two plasmids in the cell-free system allowed demonstration of functional complementation and pull-down experiments confirmed that the basic functional unit is the dimer. An additional interaction between dimers has been detected by using crosslinking between unique Cys residues. This finding implies the existence of a dimer of dimers.

L49 ANSWER 5 OF 38 MEDLINE on STN
2004212933. PubMed ID: 15111102. New insights into the structure and oligomeric state of the bacterial multidrug **transporter** EmrE: an unusual asymmetric homo-dimer. Ubarretxena-Belandia I; Tate C G. (MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.) FEBS letters, (2004 Apr 30) 564 (3) 234-8. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB EmrE is a small multidrug **transporter** that contains 110 amino acid residues that form four transmembrane alpha-helices. The three-dimensional structure of EmrE has been determined from two-dimensional crystals by electron cryo-microscopy. EmrE is an asymmetric homo-dimer with one substrate molecule bound in a chamber accessible laterally from one leaflet of the lipid bilayer. Evidence from substrate binding analyses and analytical ultracentrifugation of detergent-solubilised EmrE shows that the minimum functional unit for substrate binding is a dimer. However, it is possible that EmrE exists as a tetramer in vivo and plausible models are suggested based upon analyses of two-dimensional crystals.

L49 ANSWER 6 OF 38 MEDLINE on STN
2004151436. PubMed ID: 15044024. The membrane topology of EmrE - a small multidrug **transporter** from Escherichia coli. Ninio Shira; Elbaz Yael; Schuldiner Shimon. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) FEBS letters, (2004 Mar 26) 562 (1-3) 193-6. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB EmrE is a multidrug **transporter** from Escherichia coli that belongs to the Smr family of small multidrug **transporters.** The secondary structure of EmrE consists of a four helical bundle, as judged by different techniques. EmrE has been extensively characterized; nevertheless, the membrane topology of EmrE has not been determined yet. Previous work with a homologous Smr protein provided partial information of the membrane topology, however the location of the carboxy-terminus remained inconclusive. In this work we probed the membrane topology of EmrE, focusing on the carboxy-terminus of the protein, using two independent approaches. Our results support a secondary structure where the carboxy-terminus faces the cytoplasm, while the first loop faces the periplasm.

L49 ANSWER 7 OF 38 MEDLINE on STN
2004109623. PubMed ID: 14739638. An evaluation of detergents for NMR structural studies of membrane proteins. Krueger-Koplin Ray D; Sorgen Paul L; Krueger-Koplin Suzanne T; Rivera-Torres Ivan O; Cahill Sean M; Hicks David B; Grinius Leo; Krulwich Terry A; Girvin Mark E. (Biochemistry Department, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, U.S.A.) Journal of biomolecular NMR, (2004 Jan) 28 (1) 43-57. Journal code: 9110829. ISSN: 0925-2738. Pub. country: Netherlands. Language: English.

AB Structural information on membrane proteins lags far behind that on soluble proteins, in large part due to difficulties producing homogeneous, stable, structurally relevant samples in a membrane-like environment. In this study 25 membrane mimetics were screened using 2D (1)H-(15)N heteronuclear single quantum correlation NMR experiments to establish sample homogeneity and predict fitness for structure determination. A single detergent, 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol)] (LPPG), yielded high quality NMR spectra with sample lifetimes greater than one month for the five proteins tested - R. sphaeroides LH1 alpha and beta subunits, E. coli and B. pseudofirmus OF4 ATP synthase c subunits, and S. aureus small multidrug resistance **transporter** - with 1, 2, or 4 membrane spanning alpha-helices, respectively. Site-specific spin labeling established interhelical distances in the drug **transporter** and genetically fused dimers of c subunits in LPPG consistent with in vivo distances. Optical spectroscopy showed that LH1 beta subunits form native-like complexes with bacteriochlorophyll a in LPPG. All the protein/micelle complexes were estimated to exceed 100 kDaltons by translational diffusion measurements. However, analysis of (15)N transverse, longitudinal and (15)N[(1)H] nuclear Overhauser effect relaxation measurements yielded overall rotational correlation times of 8 to 12 nsec, similar to a 15-20 kDalton protein tumbling isotropically in solution, and consistent with the high quality NMR data observed.

L49 ANSWER 8 OF 38 MEDLINE on STN
2003197418. PubMed ID: 12590142. An amino acid cluster around the essential Glu-14 is part of the substrate- and proton-binding domain of EmrE, a multidrug **transporter** from Escherichia coli. Gutman Natalia; Steiner-Mordoch Sonia; Schuldiner Shimon. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (2003 May 2) 278 (18) 16082-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB EmrE is a small multidrug **transporter** (110 amino acids long) from Escherichia coli that extrudes various drugs in exchange with protons, thereby rendering bacteria resistant to these compounds. Glu-14 is the only charged membrane-embedded residue in EmrE and is evolutionarily highly conserved. This residue has an unusually high pK and is an essential part of the binding domain, shared by substrates and protons. The occupancy of the binding domain is mutually exclusive, and, as such, this provides the molecular basis for the coupling between substrate and proton fluxes. Systematic cysteine-scanning mutagenesis of the residues in the transmembrane segment (TM1), where Glu-14 is located, reveals an amino acid cluster on the same face of TM1 as Glu-14 that is part of the substrate- and proton-binding domain. Substitutions at most of these positions yielded either inactive mutants or mutants with modified affinity to substrates. Substitutions at the Ala-10 position, one helix turn away from Glu-14, yielded mutants with modified affinity to protons and thereby impaired in the coupling of substrate and proton fluxes. Taken as a whole, the results strongly support the concept of a common binding site for substrate and protons and stress the importance of one face of TM1 in substrate recognition, binding, and H(+)-coupled transport.

L49 ANSWER 9 OF 38 MEDLINE on STN
2003149697. PubMed ID: 12551892. Characterization of an archaeal multidrug **transporter** with a unique amino acid composition. Ninio Shira; Schuldiner Shimon. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (2003 Apr 4) 278 (14) 12000-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The Smr family of multidrug **transporters** consists of small membrane proteins that extrude various drugs in exchange with protons rendering cells resistant to these drugs. Smr proteins identified to date have been found only in Eubacteria. In this work we present the cloning and characterization of an Smr protein from the archaeon Halobacterium salinarum, the first Smr in the archaeal kingdom. The protein, named Hsmr, was identified through sequence similarity to the Smr family, and the DNA sequence was cloned into an Escherichia coli expression system. Hsmr is heterologously expressed in a functional form despite the difference in lipid composition of the membrane and the lower salt in the cell and its environment. Cells harboring the Hsmr plasmid transport ethidium bromide in an uncoupler-sensitive process and gain resistance to ethidium bromide and acriflavine. Hsmr binds tetraphenylphosphonium

(TPP(+)) with a relatively low affinity (K(D) approximately 200 nm) at low salt concentration that increases (K(D) approximately 40 nm) upon the addition of 2 m of either NaCl or KCl. The Hsmr protein contains many of the signature sequence elements of the Smr family and also a high content of negative residues in the loops, characteristic of extreme halophiles. Strikingly, Hsmr is composed of over 40% valine and alanine residues. These residues are clustered at certain regions of the protein in domains that are not important for activity, as judged from lack of conservation and from previous studies with other Smr proteins. We suggest that this high content of alanine and valine residues is a reflection of a "natural" alanine and valine scanning necessitated by the high GC content of the gene. This phenomenon reveals significant sequence elements in small multidrug **transporters**.

L49 ANSWER 10 OF 38 MEDLINE on STN

2003553913. PubMed ID: 14633977. Three-dimensional structure of the bacterial multidrug **transporter** EmrE shows it is an asymmetric homodimer. Ubarretxena-Belandia Iban; Baldwin Joyce M; Schuldiner Shimon; Tate Christopher G. (MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.) EMBO journal, (2003 Dec 1) 22 (23) 6175-81. Journal code: 8208664. ISSN: 0261-4189. Pub. country: England: United Kingdom. Language: English.

AB The small multidrug resistance family of **transporters** is widespread in bacteria and is responsible for bacterial resistance to toxic aromatic cations by proton-linked efflux. We have determined the three-dimensional (3D) structure of the Escherichia coli multidrug **transporter** EmrE by electron cryomicroscopy of 2D crystals, including data to 7.0 A resolution. The structure of EmrE consists of a bundle of eight transmembrane alpha-helices with one substrate molecule bound near the centre. The substrate binding chamber is formed from six helices and is accessible both from the aqueous phase and laterally from the lipid bilayer. The most remarkable feature of the structure of EmrE is that it is an asymmetric homodimer. The possible arrangement of the two polypeptides in the EmrE dimer is discussed based on the 3D density map.

L49 ANSWER 11 OF 38 MEDLINE on STN

2003233571. PubMed ID: 12755611. Structure of the substrate binding pocket of the multidrug **transporter** EmrE: site-directed spin labeling of transmembrane segment 1. Koteiche Hanane A; Reeves Matthew D; McHaourab Hassane S. (Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee 37232, USA.) Biochemistry, (2003 May 27) 42 (20) 6099-105. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Site-directed spin labeling (SDSL) was used to explore the structural framework responsible for the obligatory drug-proton exchange in the Escherichia coli multidrug **transporter**, EmrE. For this purpose, a nitroxide scan was carried out along a stretch of 26 residues that include transmembrane segment 1 (TMS1). This segment has been implicated in the catalytic mechanism of EmrE due to the presence of the highly conserved glutamate 14, a residue absolutely required for ligand binding. Sequence-specific variation in the accessibilities of the introduced nitroxides to molecular oxygen reveals a transmembrane helical conformation along TMS1. One face of the helix is in contact with the hydrocarbon interior of the detergent micelle while the other face appears to be solvated by an aqueous environment, resulting in significant exposure of the nitroxides along this face to NiEDDA. TMS1 from two different subunits are in close proximity near a 2-fold axis of symmetry as revealed by the analysis of spin-spin interactions at sites 14 and 18. The limited extent of spin-spin interactions is consistent with a scissor-like packing of the two TMS1. This results in a V-shaped chamber which is in contact with the aqueous phase near the N-terminus. The spatial organization of TMS1, particularly the close proximity of E14, is consistent with a proposed mechanistic model of EmrE [Yerushalmi, H., and Schuldiner, S. (2000) Biochemistry 39, 14711-14719] where substrate extrusion is coupled to proton influx through electrostatic interactions and shifts of the glutamate 14 pK(a) during the cycle.

L49 ANSWER 12 OF 38 MEDLINE on STN
2003565042. PubMed ID: 14651958. Mutagenesis of SugE, a small multidrug resistance protein. Son Mike S; Del Castilho Colin; Duncalf Karen A; Carney Dominic; Weiner Joel H; Turner Raymond J. (Department of Biological Sciences, University of Calgary, 2500 University Dr. NW, Alta., T2N 1N4, Calgary, Canada.) Biochemical and biophysical research communications, (2003 Dec 26) 312 (4) 914-21. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB The small multidrug resistance protein family has two subclasses. In this study we used a mutation approach to see what is necessary to convert a SUG subgroup member into a quaternary ammonium compound (QAC) **transporter.** We chose four key residues (H24, M39, I43, and A44) conserved within SUGs but conserved differently within the QAC **transporters.** Altogether, seven mutants were generated in Citrobacter freundii SugE. Surprisingly, the mutated SugE demonstrated an increased sensitivity to representative QACs. Additionally, ethidium uptake is found to be more prominent in the hypersensitive mutants. We conducted orientation studies using topology reporter gene fusions which indicated that SugE and the QAC **transporter** EmrE both have their N- and C-termini in the cytoplasm as predicted. The results imply that SugE can be converted to a QAC **transporter** with only a single mutation. However, because hypersensitivity was observed, the SugE mutant proteins are behaving as importers rather than as exporters.

L49 ANSWER 13 OF 38 MEDLINE on STN
2003408099. PubMed ID: 12946360. Conformational changes in the multidrug **transporter** EmrE associated with substrate binding. Tate Christopher G; Ubarretxena-Belandia Iban; Baldwin Joyce M. (MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.. cgt@mrc-lmb.cam.ac.uk) . Journal of molecular biology, (2003 Sep 5) 332 (1) 229-42. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: England: United Kingdom. Language: English.

AB EmrE is a bacterial multidrug **transporter** of the small multidrug resistance family, which extrudes large hydrophobic cations such as tetraphenylphosphonium (TPP(+)) out of the cell by a proton antiport mechanism. Binding measurements were performed on purified EmrE solubilized in dodecylmaltoside to determine the stoichiometry of TPP(+) binding; the data showed that one TPP(+) molecule bound per EmrE dimer. Reconstitution of purified EmrE at low lipid:protein ratios in either the presence or the absence of TPP(+) produced well ordered two-dimensional crystals. Electron cryo-microscopy was used to collect images of frozen hydrated EmrE crystals and projection maps were determined by image processing to 7A resolution. An average native EmrE projection structure was calculated from the c222 and p222(1) crystals, which was subsequently subtracted from the average of two independent p2 projection maps of EmrE with TPP(+) bound. The interpretation of the difference density image most consistent with biochemical data suggested that TPP(+) bound at the monomer-monomer interface in the centre of the EmrE dimer, and resulted in the movement of at least one transmembrane alpha-helix.

L49 ANSWER 14 OF 38 MEDLINE on STN
2002736582. PubMed ID: 12499164. Contributions of MexAB-OprM and an EmrE homolog to intrinsic resistance of Pseudomonas aeruginosa to aminoglycosides and dyes. Li Xian-Zhi; Poole Keith; Nikaido Hiroshi. (Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3206, USA.) Antimicrobial agents and chemotherapy, (2003 Jan) 47 (1) 27-33. Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB Of the six putative small multidrug resistance (SMR) family proteins of Pseudomonas aeruginosa, a protein encoded by the PA4990 gene (emrE(Pae)) shows the highest identity to the well-characterized EmrE efflux **transporter** of Escherichia coli. Reverse transcription-PCR confirmed the expression of emrE(Pae) in the wild-type strain of P. aeruginosa. Using isogenic emrE(Pae), mexAB-oprM, and/or mexB deletion mutants, the contributions of the EmrE protein and the MexAB-OprM efflux system to drug resistance in P. aeruginosa were assessed by a drug susceptibility test carried out in a low-ionic-strength medium, Difco nutrient broth. We found that EmrE(Pae) contributed to intrinsic resistance not only to ethidium bromide and acriflavine but also to aminoglycosides. In this low-ionic-strength medium, MexAB-OprM was also shown to contribute to aminoglycoside resistance, presumably via active efflux. Aminoglycoside resistance caused by these two pumps could not be demonstrated in high-ionic-strength media, such as Luria broth or Mueller-Hinton broth. The EmrE-dependent efflux of ethidium bromide was confirmed by a continuous fluorescence assay.

L49 ANSWER 15 OF 38 MEDLINE on STN
2002475696. PubMed ID: 12221291. Crosslinking of membrane-embedded cysteines reveals contact points in the EmrE oligomer. Soskine Misha; Steiner-Mordoch Sonia; Schuldiner Shimon. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904, Israel.) Proceedings of the National Academy of Sciences of the United States of America, (2002 Sep 17) 99 (19) 12043-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB EmrE is a small multidrug **transporter** that extrudes various drugs in exchange with protons, thereby rendering Escherichia coli cells resistant to these compounds. In this study, relative helix packing in the EmrE oligomer solubilized in detergent was probed by intermonomer crosslinking analysis. Unique cysteine replacements in transmembrane domains were shown to react with organic mercurials but not with sulfhydryl reagents, such as maleimides and methanethiosulfonates. A new protocol was developed based on the use of HgCl(2), a compound known to react rapidly and selectively with sulfhydryl groups. The reaction can bridge vicinal pairs of cysteines and form an intermolecular mercury-linked dimer. To circumvent problems inherent to mercury chemistry, a second crosslinker, hexamethylene diisocyanate, was used. After the HgCl(2) treatment, excess reagent was removed and the oligomers were dissociated with a strong denaturant. Only those previously crosslinked reacted with hexamethylene diisocyanate. Thus, vicinal cysteine-substituted residues in the EmrE oligomer were identified. It was shown that transmembrane domain (TM)-1 and TM4 in one subunit are in contact with the corresponding TM1 and TM4, respectively, in the other subunit. In addition, TM1 is also in close proximity to TM4 of the neighboring subunit, suggesting possible arrangements in the binding and translocation domain of the EmrE oligomer. This method should be useful for other proteins with cysteine residues in a low-dielectric environment.

L49 ANSWER 16 OF 38 MEDLINE on STN
2002495844. PubMed ID: 12356478. Optimization of expression and the purification by organic extraction of the integral membrane protein EmrE. Winstone Tara L; Duncalf Karen A; Turner Raymond J. (Division of Biochemistry, Department of Biological Sciences, University of Calgary, 2500 University Dr. N.W., T2N 1N4, Calgary, Alta., Canada.) Protein expression and purification, (2002 Oct) 26 (1) 111-21. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB EmrE is a member of the small multidrug resistance family of proteins and functions as a efflux **transporter** of lipophilic cations. This integral membrane protein is composed of 110 amino acids and is very hydrophobic with small loops exposed to the aqueous environment. This protein has been purified in a variety of ways including extraction with chloroform:methanol mixtures. This study explored culture growth and induction conditions, the parameters of organic solvent extraction, running conditions of a lipophilic column for lipid removal, as well as solubilization conditions. Optimal expression and purification protocols are crucial to the characterization goals of this protein. The experiments presented here led to an improvement in the yield of pure EmrE obtained by organic solvent extraction methods at a level of 0.9+/-0.2mg/L of culture. This is on the order of a 60% improvement over previous reports.

L49 ANSWER 17 OF 38 MEDLINE on STN
2001698260. PubMed ID: 11574548. Functional analysis of novel multidrug **transporters** from human pathogens. Ninio S; Rotem D; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (2001 Dec 21) 276 (51) 48250-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Proteins of the Smr family are the smallest multidrug **transporters** , about 110 amino acids long, that extrude various drugs in exchange with protons, thereby rendering bacteria resistant to these compounds. One of these proteins, EmrE, is an Escherichia coli protein, which has been cloned based on its ability to confer resistance to ethidium and methyl viologen and which has been extensively characterized. More than 60 genes coding for Smr proteins have been identified in several bacteria based on amino acid sequence similarity to the emrE gene. In this work we have analyzed the sequence similarity among these homologues and identified some distinct signature sequence elements and several fully conserved residues. Five of these homologues, from human pathogens Mycobacterium tuberculosis, Bordetella pertussis, and Pseudomonas aeruginosa and from Escherichia coli, were cloned into an E. coli expression system. The proteins were further characterized and show varying degrees of methyl viologen uptake into proteoliposomes and [(3)H]TPP binding in solubilized membranes. The homologues can also form mixed oligomers with EmrE that exhibit intermediate binding characteristics. A comparative study of various homologous proteins provides a tool for deciphering structure-function relationship and monomer-monomer interaction in multidrug **transporters** and in membrane proteins in general.

L49 ANSWER 18 OF 38 MEDLINE on STN
2001698259. PubMed ID: 11572877. In vitro monomer swapping in EmrE, a multidrug **transporter** from Escherichia coli, reveals that the oligomer is the functional unit. Rotem D; Sal-man N; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (2001 Dec 21) 276 (51) 48243-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB EmrE is a small multidrug **transporter**, 110 amino acids long that extrudes various drugs in exchange with protons, thereby rendering Escherichia coli cells resistant to these compounds. Negative dominance studies and radiolabeled substrate-binding studies suggested that EmrE functions as an oligomer. Projection structure of two-dimensional crystals of the protein revealed an asymmetric dimer. To identify the functional unit of EmrE, a novel approach was developed. In this method, quantitative monomer swapping is induced in detergent-solubilized EmrE by exposure to 80 degrees C, a treatment that does not impair transport activity. Oligomer formation is highly specific as judged by several criteria, among them the fact that (35)S-EmrE can be "pulled out" from a mixture prepared from generally labeled cells. Using this technique, we show that inactive mutant subunits are functionally complemented when mixed with wild type subunits. The hetero-oligomers thus formed display a decreased affinity to substrates. In addition, sulfhydryl reagents inhibit the above hetero-oligomer even though Cys residues are present only in the inactive monomer. It is concluded that, in EmrE, the oligomer is the functional unit.

L49 ANSWER 19 OF 38 MEDLINE on STN
2001308589. PubMed ID: 11278804. A single carboxyl mutant of the multidrug **transporter** EmrE is fully functional. Yerushalmi H; Mordoch S S; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (2001 Apr 20) 276 (16) 12744-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB EmrE, a multidrug **transporter** from Escherichia coli removes toxic compounds from the cell in exchange with protons. Glu-14 is the only charged residue in the putative membrane domains and is fully conserved in more than 50 homologues of the protein. This residue was shown to be an essential part of the binding site, common to protons and substrate. EmrE bearing a single carboxylic residue, Glu-14, shows uptake and binding properties similar to those of the wild type. This suggests that a small protein bearing only 110 amino acids with a single carboxyl in position 14 is the most basic structure that shows ion-coupled transport activity. The role of Glu-14 in substrate binding was examined by using dicyclohexylcarbodiimide, a hydrophobic carbodiimide that is known to react with carboxyls. Tetraphenylphosphonium binding to both wild type and the single carboxyl mutant is inhibited by dicyclohexylcarbodiimide in a dose-dependent manner. Ethidium and other substrates of EmrE prevent this inhibition with an order of potency in accord with their apparent affinities. This suggests that dicyclohexylcarbodiimide binding is sterically prevented by the substrate, supporting the contention that Glu-14, the reactive residue, is part of the substrate-binding site.

L49 ANSWER 20 OF 38 MEDLINE on STN

2001532913. PubMed ID: 11566977. Analysis of a complete library of putative drug **transporter** genes in Escherichia coli. Nishino K; Yamaguchi A. (Department of Cell Membrane Biology, Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567-0047, Japan.) Journal of bacteriology, (2001 Oct) 183 (20) 5803-12. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB The complete sequencing of bacterial genomes has revealed a large number of drug **transporter** genes. In Escherichia coli, there are 37 open reading frames (ORFs) assumed to be drug **transporter** genes on the basis of sequence similarities, although the transport capabilities of most of them have not been established yet. We cloned all 37 putative drug **transporter** genes in E. coli and investigated their drug resistance phenotypes using an E. coli drug-sensitive mutant as a host. E. coli cells transformed with a plasmid carrying one of 20 ORFs, i.e., fsr, mdfA, yceE, yceL, bcr, emrKY, emrAB, emrD, yidY, yjiO, ydhE, acrAB, cusA (formerly ybdE), yegMNO, acrD, acrEF, yhiUV, emrE, ydgFE, and ybjYZ, exhibited increased resistance to some of the 26 representative antimicrobial agents and chemical compounds tested in this study. Of these 20 ORFs, cusA, yegMNO, ydgFE, yceE, yceL, yidY, and ybjYZ are novel drug resistance genes. The fsr, bcr, yjiO, ydhE, acrD, and yhiUV genes gave broader resistance spectra than previously reported.

L49 ANSWER 21 OF 38 MEDLINE on STN

2001483519. PubMed ID: 11321568. Precious things come in little packages. Schuldiner S; Granot D; Steiner S; Ninio S; Rotem D; Soskin M; Yerushalmi H. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Israel.) Journal of molecular microbiology and biotechnology, (2001 Apr) 3 (2) 155-62. Ref: 55. Journal code: 100892561. ISSN: 1464-1801. Pub. country: England: United Kingdom. Language: English.

AB The 110-amino acid multidrug **transporter** from E. coli, EmrE, is a member of the family of MiniTexan or Smr drug **transporters.** EmrE can transport acriflavine, ethidium bromide, tetraphenylphosphonium (TPP+), benzalkonium and several other drugs with relatively high affinities. EmrE is an H+/drug antiporter, utilizing the proton electrochemical gradient generated across the bacterial cytoplasmic membrane by exchanging two protons with one substrate molecule. The EmrE multidrug **transporter** is unique in its small size and hydrophobic nature. Hydropathic analysis of the EmrE sequence predicts four alpha-helical transmembrane segments. This model is experimentally supported by FTIR studies that confirm the high alpha-helicity of the protein and by high-resolution heteronuclear NMR analysis of the protein structure. The TMS of EmrE are tightly packed in the membrane without any continuous aqueous domain, as was shown by Cysteine scanning experiments. These results suggest the existence of a hydrophobic pathway through which the substrates are translocated. EmrE is functional as a homo-oligomer as suggested by several lines of evidence, including co-reconstitution experiments of wild-type protein with inactive mutants in which negative dominance has been observed. EmrE has only one membrane embedded charged residue, Glu-14, that is conserved in more than fifty homologous proteins and it is a simple model system to study the role of carboxylic residues in ion-coupled **transporters.** We have used mutagenesis and chemical modification to show that Glu-14 is part of the substrate-binding site. Its role in proton binding and translocation was shown by a study of the effect of pH on ligand binding, uptake, efflux and exchange reactions. We conclude that Glu-14 is an essential part of a binding site, common to substrates and protons. The occupancy of this site is mutually exclusive and provides the basis of the simplest coupling of two fluxes. Because of some of its properties and its size, EmrE provides a unique system to understand mechanisms of substrate recognition and translocation.

L49 ANSWER 22 OF 38 MEDLINE on STN
2001387555. PubMed ID: 11443233. Small is mighty: EmrE, a multidrug **transporter** as an experimental paradigm. Schuldiner S; Granot D; Mordoch S S; Ninio S; Rotem D; Soskin M; Tate C G; Yerushalmi H. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.. shimon.schuldiner@huji.ac.il) . News in physiological sciences : an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society, (2001 Jun) 16 130-4. Ref: 18. Journal code: 8609378. ISSN: 0886-1714. Pub. country: United States. Language: English.

AB EmrE is a multidrug **transporter** from Escherichia coli that functions as a homooligomer and is unique in its small size. In each monomer there are four tightly packed transmembrane segments and one membrane-embedded charged residue. This residue provides the basis for the coupling mechanism as part of a binding site "time shared" by substrates and protons.

L49 ANSWER 23 OF 38 MEDLINE on STN
2001202782. PubMed ID: 11226157. The projection structure of EmrE, a proton-linked multidrug **transporter** from Escherichia coli, at 7 A resolution. Tate C G; Kunji E R; Lebendiker M; Schuldiner S. (MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, MRC Dunn Human Nutrition Unit, Hills Road, Cambridge CB2 2XY, UK.) EMBO journal, (2001 Jan 15) 20 (1-2) 77-81. Journal code: 8208664. ISSN: 0261-4189. Pub. country: England: United Kingdom. Language: English.

AB EmrE belongs to a family of eubacterial multidrug **transporters** that confer resistance to a wide variety of toxins by coupling the influx of protons to toxin extrusion. EmrE was purified and crystallized in two dimensions by reconstitution with dimyristoylphosphatidylcholine into lipid bilayers. Images of frozen hydrated crystals were collected by cryo-electron microscopy and a projection structure of EmrE was calculated to 7 A resolution. The projection map shows an asymmetric EmrE dimer with overall dimensions approximately 31 x 40 A, comprising an arc of highly tilted helices separating two helices nearly perpendicular to the membrane from another two helices, one tilted and the other nearly perpendicular. There is no obvious 2-fold symmetry axis perpendicular to the membrane within the dimer, suggesting that the monomers may have different structures in the functional unit.

L49 ANSWER 24 OF 38 CAPLUS COPYRIGHT 2005 ACS on STN
2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,

TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L49 ANSWER 25 OF 38 MEDLINE on STN

2001081133. PubMed ID: 11101285. A model for coupling of H(+) and substrate fluxes based on "time-sharing" of a common binding site. Yerushalmi H; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Biochemistry, (2000 Dec 5) 39 (48) 14711-9. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Both prokaryotic and eukaryotic cells contain an array of membrane transport systems maintaining the cellular homeostasis. Some of them (primary pumps) derive energy from redox reactions, ATP hydrolysis, or light absorption, whereas others (ion-coupled **transporters**) utilize ion electrochemical gradients for active transport. Remarkable progress has been made in understanding the molecular mechanism of coupling in some of these systems. In many cases carboxylic residues are essential for either binding or coupling. Here we suggest a model for the molecular mechanism of coupling in EmrE, an Escherichia coli 12-kDa multidrug **transporter.** EmrE confers resistance to a variety of toxic cations by removing them from the cell interior in exchange for two protons. EmrE has only one membrane-embedded charged residue, Glu-14, which is conserved in more than 50 homologous proteins. We have used mutagenesis and chemical modification to show that Glu-14 is part of the substrate-binding site. Its role in proton binding and translocation was shown by a study of the effect of pH on ligand binding, uptake, efflux, and exchange reactions. The studies suggest that Glu-14 is an essential part of a binding site, which is common to substrates and protons. The occupancy of this site by H(+) and substrate is mutually exclusive and provides the basis of the simplest coupling for two fluxes.

L49 ANSWER 26 OF 38 MEDLINE on STN

2001081744. PubMed ID: 11104814. Molecular properties of bacterial multidrug **transporters.** Putman M; van Veen H W; Konings W N. (Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, NL-9751 NN Haren, The Netherlands.) Microbiology and molecular biology reviews : MMBR, (2000 Dec) 64 (4) 672-93. Ref: 285. Journal code: 9706653. ISSN: 1092-2172. Pub. country: United States. Language: English.

AB One of the mechanisms that bacteria utilize to evade the toxic effects of antibiotics is the active extrusion of structurally unrelated drugs from the cell. Both intrinsic and acquired multidrug **transporters** play an important role in antibiotic resistance of several pathogens, including Neisseria gonorrhoeae, Mycobacterium tuberculosis, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, and Vibrio cholerae. Detailed knowledge of the molecular basis of drug recognition and transport by multidrug transport systems is required for the development of new antibiotics that are not extruded or of inhibitors which block the multidrug **transporter** and allow traditional antibiotics to be effective. This review gives an extensive overview of the currently known multidrug **transporters** in bacteria. Based on energetics and structural characteristics, the bacterial multidrug **transporters** can be classified into five distinct families. Functional reconstitution in liposomes of purified multidrug transport proteins from four families revealed that these proteins are capable of mediating the export of structurally unrelated drugs independent of accessory proteins or cytoplasmic components. On the basis of (i) mutations that affect the activity or the substrate specificity of multidrug **transporters** and (ii) the three-dimensional structure of the drug-binding domain of the regulatory protein BmrR, the substrate-binding site for cationic drugs is predicted to consist of a hydrophobic pocket with a buried negatively charged residue that interacts electrostatically with the positively charged substrate. The aromatic and hydrophobic amino acid residues which form the drug-binding pocket impose restrictions on the shape and size of the substrates. Kinetic analysis of drug transport by multidrug **transporters** provided evidence that these proteins may contain multiple substrate-binding sites.

L49 ANSWER 27 OF 38 MEDLINE on STN

2000105278. PubMed ID: 10637227. A membrane-embedded glutamate is required for ligand binding to the multidrug **transporter** EmrE. Muth T R; Schuldiner S. (Institute of Life Sciences, Givat Ram, Hebrew University, Jerusalem 91904, Israel.) EMBO journal, (2000 Jan 17) 19 (2) 234-40. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB EmrE is an Escherichia coli multidrug **transporter** that confers resistance to a variety of toxins by removing them in exchange for hydrogen ions. The detergent-solubilized protein binds tetraphenylphosphonium (TPP(+)) with a K(D) of 10 nM. One mole of ligand is bound per approximately 3 mol of EmrE, suggesting that there is one binding site per trimer. The steep pH dependence of binding suggests that one or more residues, with an apparent pK of approximately 7.5, release protons prior to ligand binding. A conservative Asp replacement (E14D) at position 14 of the only membrane-embedded charged residue shows little transport activity, but binds TPP(+) at levels similar to those of the wild-type protein. The apparent pK of the Asp shifts to <5.0. The data are consistent with a mechanism requiring Glu14 for both substrate and proton recognition. We propose a model in which two of the three Glu14s in the postulated trimeric EmrE homooligomer deprotonate upon ligand binding. The ligand is released on the other face of the membrane after binding of protons to Glu14.

L49 ANSWER 28 OF 38 MEDLINE on STN

2000341221. PubMed ID: 10878258. A common binding site for substrates and protons in EmrE, an ion-coupled multidrug **transporter.** Yerushalmi H; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904, Jerusalem, Israel.) FEBS letters, (2000 Jun 30) 476 (1-2) 93-7. Ref: 41. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB EmrE is an Escherichia coli 12-kDa multidrug **transporter**, which confers resistance to a variety of toxic cations by removing them from the cell interior in exchange with two protons. EmrE has only one membrane-embedded charged residue, Glu-14, that is conserved in more than 50 homologous proteins and it is a simple model system to study the role of carboxylic residues in ion-coupled **transporters.** We have used mutagenesis and chemical modification to show that Glu-14 is part of the substrate binding site. Its role in proton binding and translocation was shown by a study of the effect of pH on ligand binding, uptake, efflux and exchange reactions. We conclude that Glu-14 is an essential part of a binding site, common to substrates and protons. The occupancy of this site is mutually exclusive and provides the basis of the simplest coupling of two fluxes.

L49 ANSWER 29 OF 38 MEDLINE on STN

1999315902. PubMed ID: 10383465. Scanning cysteine accessibility of EmrE, an H+-coupled multidrug **transporter** from Escherichia coli, reveals a hydrophobic pathway for solutes. Mordoch S S; Granot D; Lebendiker M; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (1999 Jul 2) 274 (27) 19480-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language:

English.

AB EmrE is a 12-kDa Escherichia coli multidrug **transporter** that confers resistance to a wide variety of toxic reagents by actively removing them in exchange for hydrogen ions. The three native Cys residues in EmrE are inaccessible to N-ethylmaleimide (NEM) and a series of other sulfhydryls. In addition, each of the three residues can be replaced with Ser without significant loss of activity. A protein without all the three Cys residues (Cys-less) has been generated and shown to be functional. Using this Cys-less protein, we have now generated a series of 48 single Cys replacements throughout the protein. The majority of them (43) show transport activity as judged from the ability of the mutant proteins to confer resistance against toxic compounds and from in vitro analysis of their activity in proteoliposomes. Here we describe the use of these mutants to study the accessibility to NEM, a membrane permeant sulfhydryl reagent. The study has been done systematically so that in one transmembrane segment (TMS2) each single residue was replaced. In each of the other three transmembrane segments, at least four residues covering one turn of the helix were replaced. The results show that although the residues in putative hydrophilic loops readily react with NEM, none of the residues in putative transmembrane domains are accessible to the reagent. The results imply very tight packing of the protein without any continuous aqueous domain. Based on the findings described in this work, we conclude that in EmrE the substrates are translocated through a hydrophobic pathway.

L49 ANSWER 30 OF 38 MEDLINE on STN

1999121035. PubMed ID: 9922260. EmrE, a small Escherichia coli multidrug **transporter**, protects Saccharomyces cerevisiae from toxins by sequestration in the vacuole. Yelin R; Rotem D; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University, Jerusalem 91904, Israel.) Journal of bacteriology, (1999 Feb) 181 (3) 949-56. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB In this report we describe the functional expression of EmrE, a 110-amino-acid multidrug **transporter** from Escherichia coli, in the yeast Saccharomyces cerevisiae. To allow for phenotypic complementation, a mutant strain sensitive to a series of cationic lipophilic drugs was first identified. A hemagglutinin epitope-tagged version of EmrE (HA-EmrE) conferring resistance to a wide variety of drugs, including acriflavine, ethidium, methyl viologen, and the neurotoxin 1-methyl-4-phenylpyridinium (MPP+), was functionally expressed in this strain. HA-EmrE is expressed in yeast at relatively high levels (0.5 mg/liter), is soluble in a mixture of organic solvents, and can be functionally reconstituted in proteoliposomes. In bacterial cells, EmrE removes toxic compounds by active transport through the plasma membrane, lowering their cytosolic concentration. However, yeast cells expressing HA-EmrE take up 14C-methyl viologen as well as control cells do. Thus, we investigated the basis of the enhanced resistance to the above compounds. Using Cu2+ ions or methylamine, we could selectively permeabilize the plasma membrane or deplete the proton electrochemical gradients across the vacuolar membrane, respectively. Incubation of yeast cells with copper ions caused an increase in 14C-methyl viologen uptake. In contrast, treatment with methylamine markedly diminished the extent of uptake. Conversely, the effect of Cu2+ and methylamine on a plasma membrane uptake system, proline, was essentially the opposite: while inhibited by the addition of Cu2+, it remained unaffected when cells were treated with methylamine. To examine the intracellular distribution of HA-EmrE, a functional chimera between HA-EmrE and the green fluorescent protein (HA-EmrE-GFP) was prepared. The pattern of HA-EmrE-GFP fluorescence distribution was virtually identical to that of the vacuolar marker FM 4-64, indicating that the **transporter** is found mainly in this organelle. Therefore, HA-EmrE protects yeast cells by lowering the cytoplasmic concentrations through removal of the toxin to the vacuole. This novel way of detoxification has been previously suggested to function in organisms in which a large vacuolar compartment exists. This report represents the first molecular description of such a mechanism.

L49 ANSWER 31 OF 38 MEDLINE on STN
1999280180. PubMed ID: 10353713. Alpha-periodicity analysis of small multidrug resistance (SMR) efflux **transporters.** Edwards R A; Turner R J. (Department of Biological Sciences, University of Calgary, AB, Canada.) Biochemistry and cell biology = Biochimie et biologie cellulaire, (1998) 76 (5) 791-7. Journal code: 8606068. ISSN: 0829-8211. Pub. country: Canada. Language: English.

AB Proteins in the small multidrug resistance (SMR) family of transport proteins are about 110 amino acids in length and are predicted to have four transmembrane helices. This family is divided into a two groups, one of which we have referred to as small multidrug pumps (Smp) and confer resistance to a wide variety of quaternary ammonium compounds through a proton-drug efflux antiport mechanism. Members of the second group within this family have, as yet, not had their substrate profile characterized and are referred to as Sug proteins. Alpha-periodicity analysis was conducted on a set of six homologous proteins of the SMR family consisting of three established Smp and three Sug proteins. Several amino acid properties were used in the analysis including hydropathy, variability, and a substitution matrix for lipid exposed amino acids. The scanning window was varied between 8 and 14 residues and the alpha-periodicity was calculated from the peaks in the Fourier transform power spectra in the region between 3.0 and 4.3 residues/turn. This analysis adds to the hydropathy analysis to give a more confident prediction of which residues are within the lipid bilayer for each of the four transmembrane helices. Information was also obtained that allowed for the identification of zones within each transmembrane helix that face the interior of the helical bundle on one side and are lipid exposed on the other face.

L49 ANSWER 32 OF 38 MEDLINE on STN
1998351559. PubMed ID: 9688273. NMR investigation of the multidrug **transporter** EmrE, an integral membrane protein. Schwaiger M; Lebendiker M; Yerushalmi H; Coles M; Groger A; Schwarz C; Schuldiner S; Kessler H. (Institut fur Organische Chemie und Biochemie, Technische Universitat Munchen, Germany.) European journal of biochemistry / FEBS, (1998 Jun 15) 254 (3) 610-9. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB EmrE is an Escherichia coli multidrug transport protein that confers resistance to a wide range of toxicants by active transport across the bacterial cell membrane. The highly hydrophobic polytopic integral membrane protein has been purified and studied in its full-length form by high-resolution NMR spectroscopy in a mixture of chloroform/methanol/water (6:6:1, by volume). Full activity is maintained after reconstitution of the protein into proteoliposomes from this solvent mixture. A series of heteronuclear (1H-15N) two- and three-dimensional experiments, as well as triple resonance experiments, were applied to the 110-residue protein and led to the assignment of the 1H, 15N and a large part of the 13C backbone resonances as well as many of the sidechain resonances. A preliminary analysis of the secondary structure, based on sequential NOE connectivities, deviation of chemical shifts from random coil values and 3J(NH-H alpha) coupling constants supports a model where the protein forms four alpha-helices between residues 4-26 (TM1), 32-53 (TM2), 58-76 (TM3) and 85-106 (TM4). For the residues of helices TM2 and TM3 a significant line broadening occurs due to slow conformational processes.

L49 ANSWER 33 OF 38 MEDLINE on STN
97202748. PubMed ID: 9050242. EmrE, the smallest ion-coupled **transporter**, provides a unique paradigm for structure-function studies. Schuldiner S; Lebendiker M; Yerushalmi H. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Israel.. shimons@leonardo.ls.huji.ac.il) . Journal of experimental biology, (1997 Jan) 200 (Pt 2) 335-41. Ref: 36. Journal code: 0243705. ISSN: 0022-0949. Pub. country: ENGLAND: United Kingdom. Language: English.

AB EmrE is an Escherichia coli multidrug **transporter** which confers resistance to a wide variety of toxicants by actively removing them in exchange for hydrogen ions. EmrE is a highly hydrophobic 12 kDa protein which has been purified by taking advantage of its unique solubility in organic solvents. After solubilization and purification, the protein retains its ability to transport as judged from the fact that it can be reconstituted in a functional form. Hydrophobicity analysis of the sequence yielded four putative transmembrane domains of similar sizes. Results from transmission Fourier transform infrared measurements agree remarkably well with this hypothesis and yielded alpha-helical estimates of 78% and 80% for EmrE in CHCl3:MeOH and 1,2-dimyristoyl phosphocholine, respectively. Furthermore, the fact that most of the amide groups in the protein do not undergo amide-proton H/D exchange implies that most (approximately 80%) of the residues are embedded in the bilayer. These observations are only consistent with four transmembrane helices. A domain lined by Cys41 and Cys95 accessible only to substrates such as the organic mercurial 4-(chloromercuri)benzoic acid has been identified. Both residues are asymmetric in their location with respect to the plane of the membrane, Cys95 being closer than Cys41 to the outside face of the membrane. In co-reconstitution experiments of wild-type protein with three different inactive mutants, negative dominance has been observed. This phenomenon suggests that EmrE is functional as a homo-oligomer.

L49 ANSWER 34 OF 38 CAPLUS COPYRIGHT 2005 ACS on STN

1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US9491 19950801. PRIORITY: US 1994-286430 19940805.

AB Polyspecific immunoconjugates and antibody composites that bind a multidrug **transporter** protein and an antigen associated with a tumor or infectious agent are used to overcom the multidrug resistant phenotype. These immunoconjugates and composites also can be used diagnostically to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents. In example, polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen bispecific antibody were prepared for colon cancer treatment. 99mTc- and 111Indium-labeled polyspecific immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa were also prepared

L49 ANSWER 35 OF 38 MEDLINE on STN

97094864. PubMed ID: 8940098. Negative dominance studies demonstrate the oligomeric structure of EmrE, a multidrug antiporter from Escherichia coli. Yerushalmi H; Lebendiker M; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.. shimons@leonardo.los.huji.ac.il) . Journal of biological chemistry, (1996 Dec 6) 271 (49) 31044-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB EmrE, the smallest known ion-coupled **transporter**, is an Escherichia coli 12-kDa protein 80% helical and soluble in organic solvents. EmrE is a polyspecific antiporter that exchanges hydrogen ions with aromatic toxic cations such as methyl viologen. Since it is many times smaller than the classical consensus 12 transmembrane segments **transporters**, it was particularly interesting to determine its oligomeric state. For this purpose, a series of nonfunctional mutants has been generated and characterized to test their effect on the activity of the wild-type protein upon mixing. As opposed to the wild type, these mutants do not confer resistance to methyl viologen, ethidium bromide, or a series of other toxicants. Co-expression of each of the nonfunctional mutants with the wild-type protein results in a reduction in the ability of the functional **transporter** to confer resistance to several toxicants. To perform mixing experiments in vitro, all the mutants have been purified by extraction with organic solvents, reconstituted in proteoliposomes, and found to be inactive. When co-reconstituted with wild-type protein, they inhibit the activity of the latter in a dose-dependent form up to full inhibition. We assume that this inhibition is due to the formation of mixed oligomers in which the presence of one nonfunctional subunit causes full inactivation. A binomial analysis of the results based on the latter assumptions do not provide statistically significant answers but suggests that the oligomer is composed of three subunits. The results described provide the first in vitro demonstration of the functional oligomeric structure of an ion-coupled **transporter.**

L49 ANSWER 36 OF 38 MEDLINE on STN

96355480. PubMed ID: 8702890. Identification of residues in the translocation pathway of EmrE, a multidrug antiporter from Escherichia coli. Lebendiker M; Schuldiner S. (Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, 91904 Jerusalem, Israel.) Journal of biological chemistry, (1996 Aug 30) 271 (35) 21193-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB EmrE is a small, 12-kDa, highly polyspecific antiporter, which exchanges hydrogen ions with aromatic cations such as methyl viologen. EmrE-mediated transport is inhibited by the sulfhydryl-reactive reagent 4-(chloromercuri)benzoic acid (PCMB) but not by a variety of other sulfhydryl reagents. This differential effect is due to the fact that the organic mercurial is a substrate of the **transporter** and can reach domains otherwise inaccessible to the different reagents. To find out which of the three cysteine residues in EmrE is reacting with PCMB, each was replaced with serine and it was shown that none of them is essential for transport activity. A protein completely devoid of Cys residues (CL) is also capable of substrate accumulation albeit at a slower rate. Mutated proteins in which only one of the native cysteines was left whereas the other changed to serine were also constructed. The use of these proteins demonstrated that two of the three Cys in EmrE, Cys-41 and Cys-95, but not Cys-39, react with PCMB. A related mercurial, 4-(chloromercuri)benzenesulfonic acid (PCMBS), is only a very poor inhibitor, probably because of the negative charge it bears. PCMBS reacts with EmrE in an asymmetric and unique way. It reacts with the mutant bearing a single Cys residue in position 95 (CL-C95) only when the reagent is present in the outside face of the membrane and with the mutant CL-C41 only when allowed to permeate to the cell interior; as expected, it does not react with the mutant protein bearing a single Cys at position 39 (CL-C39). It is concluded that PCMB permeates through the substrate pathway of EmrE and covalently reacts with the two exposed residues, Cys-95 and Cys-41, but not with Cys-39, located on the opposite face of the helix relative to residue 41. In addition, because of the asymmetric reactivity to PCMBS, an inhibitor that does not permeate through the protein, it is concluded that Cys-41 is closer to the cytoplasmic face than Cys-95. The results demonstrate the existence of a domain accessible only to substrates and provide a unique tool for studying the substrate permeation pathway of an ion-coupled **transporter.**

L49 ANSWER 37 OF 38 MEDLINE on STN

96240342. PubMed ID: 8679552. Determining the secondary structure and orientation of EmrE, a multi-drug **transporter**, indicates a transmembrane four-helix bundle. Arkin I T; Russ W P; Lebendiker M; Schuldiner S. (Howard Hughes Medical Institute, Yale University, New Haven, Connecticut 06520, USA.) Biochemistry, (1996 Jun 4) 35 (22) 7233-8. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB EmrE is a member of a newly emerging family of MiniTEXANS, a family of multi-drug antiporters from bacteria characterized by their small size of

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     roughly 100 amino acids.  In this report we have obtained transmission
     FTIR spectra of EmrE in CHCl3:MeOH, DMPC vesicles, and Escherichia coli
     lipid vesicles.  Secondary structure analysis has shown that both in DMPC
     vesicles and in CHCl3: MeOH the protein adopts a highly helical secondary
     structure that correlates remarkably well with that predicted by
     hydropathy analysis.  The protein was shown to be resistant to amide
     proton H/D exchange, providing evidence that most of the protein is
     embedded in the lipid bilayer.  Polarized ATR-FTIR spectra of the protein
     in DMPC vesicles have shown that the helices are oriented with an average
     tilt angle of 27 degrees from the bilayer normal.  The protein was found
     to be less oriented in E. coli lipid vesicles, most likely as a result of
     the poor orientation of the bilayer lipids themselves.  Thus, the protein
     is identified as a transmembrane four-helix bundle providing valuable
     structural data for this family of multi-drug transporters.  The
     results set the stage for further studies aimed at deriving a detailed
     model for this protein.

L49  ANSWER 38 OF 38     MEDLINE on STN                   DUPLICATE 1
95204485.     PubMed ID: 7896833.   EmrE, an Escherichia coli 12-kDa multidrug
     transporter, exchanges toxic cations and H+ and is soluble in
     organic solvents. Yerushalmi H; Lebendiker M; Schuldiner S. (Division of
     Microbial and Molecular Ecology, Alexander Silberman Institute of Life
     Sciences, Hebrew University of Jerusalem, Givat Ram, Israel. ) Journal of
     biological chemistry, (1995 Mar 24) 270 (12) 6856-63.  Journal code:
     2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB   The smallest membrane protein shown to catalyze ion-coupled transport is
     documented in this report.  A gene coding for a small 110-amino acid
     membrane protein (emrE or mvrC) has been previously identified
     and cloned and shown to render Escherichia coli cells resistant to methyl
     viologen and to ethidium.  In this report, it is shown that the resistance
     is due to extrusion of the toxic compounds in a process that requires a
     proton electrochemical gradient rather than ATP.  For this purpose, cells
     in which the unc gene was inactivated were used so that the
     interconversion between the proton gradient and ATP is not possible, and
     the effect of agents, which specifically affect either of them, was tested
     on transport of ethidium in the intact cell.  In addition, EmrE has been
     overexpressed and metabolically labeled with [35S]methionine.  Strikingly,
     the protein can be quantitatively extracted with a mixture of organic
     solvents such as chloroform:methanol and is practically pure after this
     extraction.  Moreover, after addition of E. coli lipids to the
     chloroform:methanol extract, EmrE has been reconstituted in
     proteoliposomes loaded with ammonium chloride.  Upon dilution of the
     proteoliposomes in ammonium-free medium, a pH gradient was formed that
     drove transport of ethidium and methyl viologen into the proteoliposome.
     Both substrates compete with each other and exchange with previously
     transported solute.  EmrE is a multidrug transporter of a novel
     type, and, because of its size and its solubility properties, it provides
     a unique model to study structure-function aspects of transport reactions
     in ion-coupled processes.


=> s l11 and "MsrA"
L50         37 L11 AND "MSRA"

=> dup remove l50
PROCESSING COMPLETED FOR L50
L51           19 DUP REMOVE L50 (18 DUPLICATES REMOVED)

=> d l51 1-19 cbib abs

L51  ANSWER 1 OF 19  CAPLUS  COPYRIGHT 2005 ACS on STN
2003:931485   Document No. 140:1654  Genes involving regulation of
     Staphylococcus aureus biofilm formation and antibiotic resistance.
     Yorgey, Peter S.; Chien, Yueh-tyng; Bryant, Alexander; Walbridge, Michael
     J.; Danese, Paul (Microbia, Inc., USA).  PCT Int. Appl. WO 2003097813 A2
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20031127, 67 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US15946 20030519. PRIORITY: US 2002-PV381726 20020517.

AB The present invention relates to the staphylococcus aureus genes involved in biofilm formation and antibiotic resistance. These genes, their transcriptional regulatory regions and gene products may be used for regulating biofilm formation or increasing sensitivity to antimicrobial agents as well as identifying compds. that are useful in com. important fields such as manufacturing and health care.

L51 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2005 ACS on STN

2003:570524 Document No. 139:112777 A minimal genome for an autonomous prototrophic or auxotrophic organism. Evans, Glen A. (USA). U.S. Pat. Appl. Publ. US 2003138777 A1 20030724, 170 pp. (English). CODEN: USXXCO. APPLICATION: US 2001-960858 20010920.

AB A minimal genome for a viable autonomous prototrophic or auxotrophic organism is described. The minimal gene set encodes functions including transcription, translation, aerobic metabolism, glycolysis/pyruvate dehydrogenase/pentose phosphate pathways, carbohydrate metabolism, central intermediary metabolism, nucleotide metabolism, transport and binding proteins, and housekeeping functions. Functional categories can be arranged in a predetd. phys. or temporal order. A prototrophic basic genetic operating system sufficient for autonomous viability can contain a minimal gene set of about 152 or less fundamental genes, orthologs or nonothorologous displacements thereof. An auxotrophic basic genetic operating system sufficient for autonomous viability in the presence of an auxotrophic biomol. can contain about 151 or less fundamental genes, orthologs or nonothorologous displacements thereof. Also provided is a basic genetic operating system sufficient for autonomous prototrophic or auxotrophic viability which can have an expression control region for the production of a biomol. Viable autonomous prototrophic and auxotrophic nanomachines are also provided.

L51 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2005 ACS on STN

2003:920687 Document No. 140:123489 Genetic analysis of a pathogenic Erwinia sp. isolated from pear in Japan. Maxson-Stein, Kimberly; McGhee, Gayle C.; Smith, James J.; Jones, Alan L.; Sundin, George W. (Department of Plant Pathology, Michigan State University, East Lansing, MI, 48824, USA). Phytopathology, 93(11), 1393-1399 (English) 2003. CODEN: PHYTAJ. ISSN: 0031-949X. Publisher: American Phytopathological Society.

AB Four Erwinia strains, originally isolated in Japan from pear trees with bacterial shoot blight symptoms, were analyzed to determine their genetic relationship with Erwinia amylovora and E. pyrifoliae. When genomes were characterized with amplified fragment length polymorphism markers and by comparative groEL sequence anal., the Japanese Erwinia sp. and South Korean E. pyrifoliae strains were placed in the same group, which was phylogenetically distinct from a group of 15 strains of E. amylovora. Sequencing of the 29,593-bp plasmid pEJ30 from Erwinia strain Ejp556 revealed that this plasmid was nearly identical to plasmid pEP36 from E. pyrifoliae and was closely related to the nontransferable ubiquitous plasmid pEA29 from E. amylovora. Twenty-one presumptive genes and their order in pEP36 were highly conserved in pEJ30. However, transposon Tn5394, which was present in pEP36, was not found in pEJ30. Short-sequence DNA repeats were conserved between pEJ30 and pEP36, and were different from short-sequence repeats in pEA29. Despite base-pair mismatches, primer pairs used in pEA29 polymerase chain reaction assays for E. amylovora amplified plasmid DNA from the Japanese Erwinia Ejp556 and Ejp562. Like E. pyrifoliae and a few strains of E. amylovora, Japanese Erwinia Ejp617 contained plasmids related to E. pyrifoliae

ColE1-related plasmid pEP2.6. Based on these genetic analyses, we conclude that the Erwinia pathogen of pear in Japan is closely related to E. pyrifoliae and that both of these pathogens are demonstrably distinct from E. amylovora.

L51 ANSWER 4 OF 19 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 1

2003:235637 Document No.: PREV200300235637. Characteristic expression of three genes, msr(A), mph(C) and erm(Y), that confer resistance to macrolide antibiotics on Staphylococcus aureus. Matsuoka, Mayumi [Reprint Author]; Inoue, Matsuhisa; Endo, Yoshihiro; Nakajima, Yoshinori. Division of Microbiology, Hokkaido College of Pharmacy, 7-1 Katsuraoka-cho, Otaru, Hokkaido, 047-0264, Japan. matsuoka@hokuyakudai.ac.jp. FEMS Microbiology Letters, (28 March 2003) Vol. 220, No. 2, pp. 287-293. print. CODEN: FMLED7. ISSN: 0378-1097. Language: English.

AB We have reported that the gene mph(C) (formally referred to as 'mphBM') is located on plasmid pMS97 342 bp downstream of the msr(A) gene. msr(A) specifies resistance to macrolides by ABC-**transporter**-mediated efflux, and mph(C) has 49% identity to the amino acid sequence of MPH(2')II, which encodes a phosphotransferase that inactivates some macrolide antibiotics. A strain of Staphylococcus aureus NCTC8325 containing plasmid pMS97 inactivated unlabeled and 14C-labeled erythromycin when tested by bioautographic and radioautographic techniques. In addition to erythromycin, other 14-membered ring macrolides (except for ketolides), 15-membered ring macrolides and 16-membered ring macrolides, mycinamicin, rosamicin and YM133, were inactivated by the strain. Erythromycin inactivation products produced by the strain carrying pMS97 were completely different from those produced by Escherichia coli BM694 bearing plasmid pAT63, which contains the ereA gene encoding an esterase that hydrolyzes macrolide lactones. Constructs formed with the msr(A) and mph(C) genes, and with the msr(A), mph(C) and erm(Y) genes, showed erythromycin-inactivating activity, but another construct built with the mph(C) gene alone failed to show such activity. This result suggests that any region of the msr(A) gene is needed for the expression of mph(C).

L51 ANSWER 5 OF 19 MEDLINE on STN DUPLICATE 2

2003438289. PubMed ID: 13678826. Msr(A) and related macrolide/streptogramin resistance determinants: incomplete **transporters**?. Reynolds Elinor; Ross Jeremy I; Cove Jonathan H. (Division of Microbiology, School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK.) International journal of antimicrobial agents, (2003 Sep) 22 (3) 228-36. Journal code: 9111860. ISSN: 0924-8579. Pub. country: Netherlands. Language: English.

AB The gene msr(A) confers inducible resistance to 14-membered-ring macrolides and type B streptogramins (MS(B) resistance) in staphylococci. The encoded hydrophilic protein (Msr(A)) is 488 amino acids and contains two ATP-binding motifs characteristic of the ABC **transporters**. The classical organisation of ABC **transporters** requires interaction between the two cytoplasmically located ATP-binding domains with two hydrophobic domains positioned in the membrane. Msr(A) appears to mediate drug efflux and yet contains no hydrophobic membrane spanning domains. In addition, Msr(A) functions in previously sensitive heterologous hosts such as Staphylococcus aureus in the absence of other plasmid encoded products. Current research on Msr(A) and related determinants in Gram-positive cocci and in antibiotic producing organisms is reviewed. Alternative hypotheses for the mechanism of action of Msr(A) (i.e. active transport vs. ribosomal protection) are discussed. Evidence indicating Msr(A) may have a role in virulence in addition to conferring antibiotic resistance is also considered.

L51 ANSWER 6 OF 19 MEDLINE on STN DUPLICATE 3

2002730736. PubMed ID: 12493782. A novel inhibitor of multidrug efflux pumps in Staphylococcus aureus. Gibbons Simon; Oluwatuyi Moyosoluwa; Kaatz Glenn W. (Centre for Pharmacognosy and Phytotherapy, The School of

Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK.. simon.gibbons@ulsop.ac.uk) . Journal of antimicrobial chemotherapy, (2003 Jan) 51 (1) 13-7. Journal code: 7513617. ISSN: 0305-7453. Pub. country: England: United Kingdom. Language: English.

AB GG918, a synthetic inhibitor of P-glycoprotein-mediated mammalian tumour multidrug resistance, was found to be equipotent to reserpine in enhancing the in vitro activity of norfloxacin and ciprofloxacin against strains of Staphylococcus aureus expressing distinct efflux-related multidrug resistance pumps. Four- to eight-fold reductions in MICs of these fluoroquinolones were observed for SA-1199B, a strain that overexpresses NorA (the major S. aureus multidrug **transporter**), and SA-K2068, which possesses a multidrug efflux-related pump distinct from NorA. Neither inhibitor potentiated the activity of newer fluoroquinolones such as levofloxacin or moxifloxacin by more than two-fold, and this effect was observed only in SA-1199B and SA-K2068. GG918 and reserpine exposure resulted in two- to four-fold reductions in norfloxacin and ciprofloxacin MICs in a fluoroquinolone-susceptible control strain and in strains expressing the **MsrA** and TetK proteins, which mediate efflux-related resistance to macrolides and tetracyclines, respectively, suggesting inhibition of as yet uncharacterized pumps for which norfloxacin and ciprofloxacin are substrates. In the **MsrA**- and TetK-expressing strains no more than a two-fold augmentation of erythromycin or tetracycline activity was observed with either inhibitor, suggesting minimal, if any, inhibitory activity against these efflux proteins. Using GG918 as a lead compound, a structure-activity evaluation may reveal a more potent and broader spectrum inhibitor of S. aureus antibiotic efflux pumps.

L51 ANSWER 7 OF 19 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2003:517532 Document No.: PREV200300520020. Construction and characterization of insertion/deletion mutations of plant up-regulated genes and HMM predicted hrp regulated genes in Erwinia chrysanthemi 3937. Yang, S. [Reprint Author]; Shi, X. [Reprint Author]; Lonardi, S.; Perna, N. T.; Cooksey, D. A. [Reprint Author]; Keen, N. T. [Reprint Author]; Yang, C. [Reprint Author]. Center for Plant Cell Biology, University of California, Riverside, CA, USA. Abstracts of the General Meeting of the American Society for Microbiology, (2003) Vol. 103, pp. B-294. http://www.asmusa.org/mtgsrc/generalmeeting.htm. cd-rom. Meeting Info.: 103rd American Society for Microbiology General Meeting. Washington, DC, USA. May 18-22, 2003. American Society for Microbiology. ISSN: 1060-2011 (ISSN print). Language: English.

AB Erwinia chrysanthemi is an opportunistic plant pathogen that causes soft-rot, wilts and blight diseases on a wide range of plant species. Using a GFP based IVET leaf assay, 61 confirmed plant up-regulated clones have been identified from ca. 10,000 clones screened. Plant up-regulated genes include already known or new putative virulence factors (dspA, hrpA/hrpB, ogl, **msrA**, indA, crp, togT, outF/outG, and iaaM), transcriptional regulators (crp, phoB, metR, srpS, xylR, cheR, and relB), iron uptake systems (yfeA and hmuS/hmuT/hmuU), chemotaxis systems (cheR and trg), **transporters**/permeases (hrpA/hrpB, outF/outG, togT, yfeA, and hmuS/hmuT/hmuU), xenobiotic resistance determinants (bacA and crcB), and reactive oxygen stress responses systems (**msrA**, indA, and metR). By using known and hrp functional cloning project-identified hrp promoter sequences as a training set, a hidden Markov model (HMM) was developed for a genome-wide prediction of hrp regulated promoters in E. chrysanthemi 3937. These predicted hrp regulated promoters were further confirmed by our hrp functional screening system. To further identify the virulence roles of these plant or hrp cluster up-regulated genes in Erwinia chrysanthemi 3937, Tn-5 mutagenesis and crossover PCR null knockout approaches have been used to construct the mutants. Thus far, eleven Tn5 insertion mutants of plant or hrp cluster up-regulated genes and the genes flanking them were constructed and their virulence was tested on African violet leaves (Rosalie cultivar). Among these, mutants of E. coli purM homologue, as well as hrpB, hrcJ, and hrpD homologues from

Erwinia carotovorum hrpA operon reduced virulence dramatically. The phenotypes of hrpB, hrcJ, and hrpD mutants further confirmed our previously results that hrp genes are crucial virulence determinants in E. chrysanthemi 3937.

L51 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2005 ACS on STN
2002:932867 Document No. 138:218059 Relatedness of chromosomal and plasmid DNAs of Erwinia pyrifoliae and Erwinia amylovora. McGhee, Gayle C.; Schnabel, Elise L.; Maxson-Stein, Kimberly; Jones, Beatrix; Stromberg, Verlyn K.; Lacy, George H.; Jones, Alan L. (Department of Plant Pathology, Michigan State University, East Lansing, MI, 48824-1312, USA). Applied and Environmental Microbiology, 68(12), 6182-6192 (English) 2002. CODEN: AEMIDF. ISSN: 0099-2240. Publisher: American Society for Microbiology.

AB The plant pathogen Erwinia pyrifoliae has been classified as a sep. species from Erwinia amylovora based in part on differences in mol. properties. In this study, these and other mol. properties were examined for E. pyrifoliae and for addnl. strains of E. amylovora, including strains from brambles (Rubus spp.). The nucleotide composition of the internal transcribed spacer (ITS) region was determined for six of the seven 16S-23S rRNA operons detected in these species with a 16S rRNA gene probe. Each species contained four operons with a tRNAGlu gene and two with tRNAIle and tRNAAla genes, and anal. of the operons from five strains of E. amylovora indicated a high degree of ITS variability among them. One tRNAGlu-containing operon from E. pyrifoliae Ep1/96 was identical to one in E. amylovora Ea110, but three tRNAGlu operons and two tRNAIle and tRNAAla operons from E. pyrifoliae contained unique nucleotide changes. When groEL sequences were used for species-specific identification, E. pyrifoliae and E. amylovora were the closest phylogenetic relatives among a set of 12 bacterial species. The placement of E. pyrifoliae distinct from E. amylovora corroborated mol. hybridization data indicating low DNA-DNA similarity between them. Determination of the nucleotide sequence of plasmid pEP36 from E. pyrifoliae Ep1/96 revealed a number of presumptive genes that matched genes previously found in pEA29 from E. amylovora and similar organization for the genes and origins of replication. Also, pEP36 and pEA29 were incompatible with clones containing the reciprocal origin regions. Finally, the ColE1-like plasmid pEP2.6 from strain Ep1/96 contained sequences found in small plasmids in E. amylovora strains IL-5 and IH3-1.

L51 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2005 ACS on STN
2001:392741 Document No. 136:97126 Transcript expression in Saccharomyces cerevisiae at high salinity. Yale, Jaqueline; Bohnert, Hans J. (Department of Biochemistry, University of Arizona, Tucson, AZ, 856721-0088, USA). Journal of Biological Chemistry, 276(19), 15996-16007 (English) 2001. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Transcript expression of Saccharomyces cerevisiae at high salinity was determined by microarray anal. of 6144 open reading frames (ORFs). From cells grown in 1 M NaCl for 10, 30, and 90 min, changes in transcript abundance >2-fold were classified. Salinity-induced ORFs increased over time: 107 (10 min), 243 (30 min), and 354 (90 min). Up-regulated, functionally unknown ORFs increased from 17 to 149 over this period. Expression patterns were similar early, with 67% of up-regulated transcripts after 10 min identical to those at 30 min. The expression profile after 90 min revealed different up-regulated transcripts (identities of 13% and 22%, resp.). Nucleotide and amino acid metabolism exemplified the earliest responses to salinity, followed by ORFs related to intracellular transport, protein synthesis, and destination. Transcripts related to energy production were up-regulated throughout the time course with respiration-associated transcripts strongly induced at 30 min. Highly expressed at 90 min were known salinity stress-induced genes, detoxification-related responses, **transporters** of the major facilitator superfamily, metabolism of energy reserves, nitrogen and sulfur compds., and lipid, fatty acid/isoprenoid biosynthesis. We chose severe stress conditions to monitor responses in essential biochem. mechanisms.

In the mutant, Δgpdl/gpd2, lacking glycerol biosynthesis, the stress response was magnified with a partially different set of up-regulated ORFs.

L51 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2005 ACS on STN

2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L51 ANSWER 11 OF 19 MEDLINE on STN DUPLICATE 4

2000187157. PubMed ID: 10722498. Macrolide resistance genes in Enterococcus spp. Portillo A; Ruiz-Larrea F; Zarazaga M; Alonso A; Martinez J L; Torres C. (Area Bioquimica y Biologia Molecular, Universidad de La Rioja, 26004 Logrono, Spain.) Antimicrobial agents and chemotherapy, (2000 Apr) 44 (4) 967-71. Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB Seventy-eight isolates of different Enterococcus species (E. faecalis, n = 27; E. faecium, n = 23; E. durans, n = 8; E. avium, n = 6; E. hirae, n = 9; E. gallinarum, n = 3; and E. casseliflavus, n = 2) with a variety of erythromycin resistance phenotypes were examined for the presence of macrolide resistance genes (ermA, ermB, ermC, ermTR, mefA/E, and **msrA**). Positive PCR amplifications of ermB were obtained for 39 of 40 highly erythromycin-resistant Enterococcus isolates (MICs, >128 microg/ml) of different species; the remaining highly resistant E. faecium isolate was positive for PCR amplification of ermA but was negative for PCR amplification of the ermB and ermC genes. For all enterococcal strains for which erythromycin MICs were < or =32 microg/ml PCRs were negative for erm methylase genes. For all E. faecium isolates PCR amplified products of the expected size of 400 bp were obtained when **msrA** primers were used, with the results being independent of the erythromycin resistance phenotype. All the other enterococcal species gave negative results by **msrA** PCRs. Sequencing of the **msrA** PCR products from either erythromycin-susceptible, low-level-resistant, or highly resistant E. faecium strains showed that the amplicons did not correspond to the **msrA** gene described for Staphylococcus epidermidis but corresponded to a new putative efflux determinant, which showed 62% identity with the **msrA** gene at the DNA level and 72% similarity at the amino acid level. This new gene was named msrC.

L51 ANSWER 12 OF 19 MEDLINE on STN DUPLICATE 5

2000236074. PubMed ID: 10774259. Study of macrolide, lincosamide, and streptogramin B antibiotics resistance in Staphylococcus aureus. Matsuoka M. (Division of Microbiology, Hokkaido College of Pharmacy, Japan.) Yakugaku zasshi. Journal of the Pharmaceutical Society of Japan, (2000 Apr) 120 (4) 374-86. Ref: 48. Journal code: 0413613. ISSN: 0031-6903. Pub. country: Japan. Language: Japanese.

AB Macrolide antibiotics (Mac) consist of a 12- to 16-membered lactone ring combined with a sugar moiety, and they inhibit protein synthesis via binding to 23S ribosomal RNA in bacteria. The 14- and 16-membered Mac are used for treating infectious diseases caused by Gram-positive and other bacteria; e.g., Haemophilus influenzae, Bordetella pertussis, Legionella pneumophila, Campylobacter, Treponema pallidum and Mycoplasma. Resistance to macrolide, lincosamide, and streptogramin-B (MLS) antibiotics in staphylococci is known to have the following mechanisms: 1) alteration of the target on ribosome due to dimethylation of a specific adenine residue in the 23S ribosomal RNA by the product of the erm gene, and consequently a decrease in binding of MLS antibiotics; 2) inactivation of streptogramin-B (STG-B) and lincosamide by the products of the sbh (encoding streptogramin B hydrolase) and linA' (encoding 3-lincomycin 4-clindamycin O-nucleotidyltransferase) genes, respectively; and 3) active efflux of Mac and STG-B antibiotics determined by the **msrA** and msrB genes in Staphylococcus epidermidis and Staphylococcus xylosus, respectively, both of which appear to act as an ATP-dependent efflux pump. I have shown that Staphylococcus aureus 8325(pEP2104) exhibits inducible resistance to PMS (partial macrolide and streptogramin B)-antibiotics [the 14-membered macrolides, erythromycin (EM), and oleandomycin (OL), and the 16-membered macrolide mycinamicin (MCM) and STG-B]. The sequence of the N-terminal amino acid residues of a 63 kDa protein (MsrSA) that appeared in the membrane of PMS-resistant strains was identical to that of an **MsrA** polypeptide related to enhanced efflux of [14C]EM. Ribosomes from PMS-resistant strains showed a similar affinity for EM to those from the PMS-sensitive host strain NCTC8325, and no inactivation of EM by 8325(pEP2104) was observed. In the present study, I showed the DNA sequence of the msrSA region on the constitutive PMS-resistant plasmid pMC38, PMS-inducible resistant plasmid pEP2104 and PMS-sensitive mutant plasmid pSP6, and the region that is essential for inducible expression in PMS resistance. In addition, I investigated the relationship between PMS resistance and intracellular accumulation of EM.

L51 ANSWER 13 OF 19 CAPLUS COPYRIGHT 2005 ACS on STN
1999:734683 Document No. 132:216310 Resistance to macrolides mediated by efflux mechanisms. Sutcliffe, Joyce (Department of Infectious Diseases, Pfizer Central Research Inc, Groton, CT, 06340, USA). Current Opinion in Anti-Infective Investigational Drugs, 1(4), 403-412 (English) 1999. CODEN: COADFY. ISSN: 1464-8458. Publisher: Current Drugs Ltd..

AB A review with 92 refs. Drug transport proteins can be divided into two classes: secondary **transporters**, which couple extrusion of drugs with ion exchange, and ATP-binding cassette (ABC) **transporters**, which utilize the energy of a phosphate bond upon ATP hydrolysis to efflux drugs. Efflux is mediated by either a pump with narrow specificity, e.g, Mef and **MsrA**, or a pump with broad specificity, e.g., AcrAB-TolC, AcrEF-TolC, MexAB-OprM, MexCD-OprJ, AmrAB-OprA, MtrCDE, EmrE, MdfA and Cmr. Staphylococcus epidermidis harbors a plasmid with an **msrA** determinant, an ABC-type **transporter** specific for 14- and 15- membered macrolides and streptogramin B. An almost identical gene resides in Staphylococcus aureus (S aureus), although it appears to be uncommon. **MsrA** appears to be the ATP binding component of the ABC **transporter** and likely works in concert with various pumps that have been identified on plasmids or on the chromosome of staphylococcal strains. MsrB, found in Staphylococcus xylosus, is identical to the C-terminal portion of **MsrA** and likely functions as a dimer. MefA and MefE, initially described in Streptococcus pyogenes and Streptococcus pneumoniae, resp., have been folded into a single family, MefA, because the two genes are approx. 90% identical at the amino acid level. The MefA protein is a member of the major facilitator superfamily (MFS) and can efflux 14- and 15- membered macrolides. Resistance to 16-membered macrolides is not seen even in the presence of inducing concns. of erythromycin. Strains harboring the mefA gene remain susceptible to streptogramin B and clindamycin. Interestingly, the MefA protein conferred macrolide resistance when cloned into Escherichia coli (E coli). More recently, mefA has been described in many different streptococcal species, moderately erythromycin-resistant strains of Enterococcus faecium, Micrococcus luteus and Corynebacterium jeikeiium. Other members of the MFS class that can cause the active egress of erythromycin include MdfA in E coli and Cmr in Corynebacterium glutamicum. The latter two require overexpression to confer approx. 4-fold level of resistance. Another putative pump, MreA, was described in a clin. strain of Streptococcus agalactiae, but recent work demonstrates that this protein likely encodes a riboflavin kinase (ribC). The AcrAB-TolC pump is found in E coli and similar members of the resistance-nodulation-cell division family appear to be present in Haemophilus influenzae (AcrAB-like), Pseudomonas aeruginosa (MexAB-OprM, MexCD-OprJ), Burkholderia pseudomallei (AmrAB-OprA) and Neisseria gonorrhoeae (MtrCDE). The **transporters** in the Gram-neg. bacteria (AcrB, AcrF, MexB, MexD, AmrB, MtrD) work in concert with an accessory protein (AcrA, AcrE, MexA, MexC, AmrA, MtrC) which spans the periplasmic space and interacts with a porin in the outer membrane (TolC, TolC(), OprM, OprJ, OprA, MtrE). AcrAB-TolC and MtrCDE confer resistance to hydrophobic antibiotics (including erythromycin), detergents and dyes, while the two multidrug pumps from Pseudomonas overlap in their ability to efflux compds. such as tetracycline, novobiocin, chloramphenicol, erythromycin and fluoroquinolones, but show specificity toward certain β-lactams and fourth generation cephalosporins. AmrAB-OprA confers high-level resistance to aminoglycosides and macrolides. Some of these pumps are constitutive, e.g., AcrAB-TolC, AcrAB-Omp2, MexAB-OprM, AmrAB-OprA, while others remain silent until promoter or regulatory mutations allow their expression. Macrolide resistance can also be conferred by members of the small multidrug resistance family, EmrE and Mmr. EmrE is chromosomally encoded in E coli and has no known transmembrane linker or outer membrane channel associated with it. Reconstituted EmrE catalyzes the efflux or erythromycin and tetracycline, in addition to lipophilic monovalent cations. Mmr has been cloned from Mycobacterium tuberculosis and confers erythromycin resistance in Mycobacterium smegmatis. All the resistance determinants are intrinsic and chromosomally encoded, with the exception of **msrA** and mefA. Recently, mefA was transferred to naive strains by conjugation; it is likely mobile by a chromosomally integrated transposon. **MsrA** is generally plasmid-mediated. The expression of both genes is inducible by erythromycin.

L51 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2005 ACS on STN
1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US9491 19950801. PRIORITY: US 1994-286430 19940805.

AB Polyspecific immunoconjugates and antibody composites that bind a multidrug **transporter** protein and an antigen associated with a tumor or infectious agent are used to overcom the multidrug resistant phenotype. These immunoconjugates and composites also can be used diagnostically to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents. In example, polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen bispecific antibody were prepared for colon cancer treatment. 99mTc- and 111Indium-labeled polyspecific immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa were also prepared

L51 ANSWER 15 OF 19 MEDLINE on STN DUPLICATE 6
97149291. PubMed ID: 8996099. Minimal functional system required for expression of erythromycin resistance by **msrA** in Staphylococcus aureus RN4220. Ross J I; Eady E A; Cove J H; Baumberg S. (Department of Microbiology, University of Leeds, UK.) Gene, (1996 Dec 12) 183 (1-2) 143-8. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands.

Language: English.

AB Previous studies have suggested that inducible erythromycin (Er) resistance in staphylococci mediated by the plasmid-borne ABC-**transporter msrA** is dependent on additional unidentified chromosomally encoded transmembrane (TM) domains. The requirement for two S. aureus candidate sequences, stpC and smpC, highly similar to sequences adjacent to **msrA** on the original S. epidermidis plasmid was investigated. Deletion of the sequences by allelic replacement was accomplished by electroporation of S. aureus RN4220 with a nonreplicating suicide vector. S. aureus strains carrying a delta(stpC-smpC) mutation showed an identical ErR phenotype to those arising from single crossover events and unmutated RN4220 containing **msrA.** This proves that neither stpC nor smpC is required for ErR. To further define the minimal functional unit required for MSR, the control region within the leader sequence of **msrA** was deleted. This resulted in constitutive resistance to Er and type B streptogramins (Sg), proving that SgR does not require the presence of Er. Deletion constructs containing the N- or C-terminal ABC regions of **MsrA** did not confer ErR in RN4220 singly or in combination.

L51 ANSWER 16 OF 19 MEDLINE on STN DUPLICATE 7

95189112. PubMed ID: 7883194. Identification of a chromosomally encoded ABC-transport system with which the staphylococcal erythromycin exporter **MsrA** may interact. Ross J I; Eady E A; Cove J H; Baumberg S. (Department of Microbiology, University of Leeds, UK.) Gene, (1995 Feb 3) 153 (1) 93-8. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB The energy-dependent efflux of erythromycin (Er) in staphylococci is due to the presence of msr A, which encodes an ATP-binding protein. **MsrA** is related to the multi-component ATP-binding cassette (ABC) **transporters** which characteristically also contain membrane-spanning domains. Since **MsrA** functions in a heterologous host in the absence of other plasmid-encoded products, the requirement for a transmembrane (TM) complex might be fulfilled by hijacking a chromosomally encoded protein. Two genes, stpA and smpA, were identified upstream from **msrA** on the original Staphylococcus epidermidis plasmid, encoding an ATP-binding protein and a hydrophobic TM protein, respectively. Sequences highly similar to stpA and smpA (stpB and smpB) were also found adjacent to a chromosomal copy of **msrA** in S. hominis. In Southern blots, internal fragments of stpA or smpA hybridized to the chromosome of the Ers S. aureus RN4220. Cloning and sequence analysis of the region identified revealed the presence of two genes, stpC and smpC, related to stpA and smpA. The deduced amino-acid sequences of the gene products showed that StpA and StpC were 85% identical, whereas SmpA and SmpC were 65% identical. A gene similar to **msrA** was not present in the S. aureus chromosome. There was no further sequence similarity outside these conserved regions. These results indicate that the chromosomes of S. hominis and S. aureus contain sequences encoding a potential TM protein with which **MsrA** might interact.

L51 ANSWER 17 OF 19 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN DUPLICATE 8

1995:103983 Document No.: PREV199598118283. Expression and functional studies of **MSRA**, an ABC **transporter** mediating antibiotic resistance in staphylococci. Ross, J. I. [Reprint author]; Eady, E. A. [Reprint author]; Cove, J. H. [Reprint author]; Cunliffe, W. J.. Dep. Microbiol., Univ. Leeds, Leeds LS2 9JT, UK. Zentralblatt fuer Bakteriologie Supplement, (1994) Vol. 26, No. 0, pp. 179-181. ISSN: 0941-018X. Language: English.

L51 ANSWER 18 OF 19 MEDLINE on STN

93316854. PubMed ID: 8326867. Streptomyces antibioticus contains at least three oleandomycin-resistance determinants, one of which shows similarity with proteins of the ABC-**transporter** superfamily. Rodriguez A M;

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     Olano C; Vilches C; Mendez C; Salas J A. (Departamento de Biologia
     Funcional, Universidad de Oviedo, Spain. ) Molecular microbiology, (1993
     May) 8 (3) 571-82.  Journal code: 8712028. ISSN: 0950-382X. Pub. country:
     ENGLAND: United Kingdom. Language: English.
AB   Three different DNA fragments of an oleandomycin producer, Streptomyces
     antibioticus, conferring oleandomycin resistance were cloned in plasmid
     pIJ702 and expressed in Streptomyces lividans and in Streptomyces albus.
     These oleandomycin resistance determinants were designated as oleA
     (pOR400), oleB (pOR501) and oleC (pOR800). oleA and oleC are closely
     linked in the chromosome as they were both obtained together in two cosmid
     clones that were isolated from a genomic library.  Sequencing of the oleC
     resistance determinant revealed four complete open reading frames (ORFs)
     and the C-terminal end of a fifth.  The functions of orf1 and orf2 are
     unknown since they did not show significant similarity with other
     sequences in the data bases.  The orf3 gene product has similarity with
     some proteins involved in iron and vitamin B12 uptake in bacteria.  The
     orf4 gene product had a hydrophilic profile and showed important
     similarity with proteins containing typical ATP-binding domains
     characteristic of the ABC-transporter superfamily and involved
     in membrane transport and, particularly, with several genes conferring
     resistance to various macrolide antibiotics and anticancer drugs.  The
     last gene, orf5, is translationally coupled to orf4 and codes for a
     hydrophobic polypeptide containing several transmembrane domains
     characteristic of integral membrane proteins.  Subcloning and deletion
     experiments limited the resistance determinant to a 0.9 kb PstI-SphI
     fragment and only orf4 is included in this fragment.  These results
     suggest that resistance to oleandomycin conferred by oleC (orf4) is
     probably due to an efflux transport system of the ABC-transporter
     superfamily.

L51  ANSWER 19 OF 19  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation.
     on STN
92:234217   The Genuine Article (R) Number: HL846. EXPRESSION AND
     FUNCTIONAL-STUDIES OF MSRA, AN ABC TRANSPORTER
     MEDIATING ANTIBIOTIC-RESISTANCE IN STAPHYLOCOCCI.  ROSS J I (Reprint);
     COVE J H; CUNLIFFE W J. UNIV LEEDS, DEPT MICROBIOL, LEEDS LS2 9JT, W
     YORKSHIRE, ENGLAND; GEN INFIRM, DEPT DERMATOL, LEEDS LS1 3EX, W YORKSHIRE,
     ENGLAND. JOURNAL OF INVESTIGATIVE DERMATOLOGY (APR 1992) Vol. 98, No. 4,
     pp. 521. ISSN: 0022-202X. Pub. country: ENGLAND. Language: ENGLISH.


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L52          19 L11 AND "DRRA"

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L53  ANSWER 1 OF 10     MEDLINE on STN                    DUPLICATE 1
2004305775.    PubMed ID: 15090538.   Membrane topology of the DrrB protein of
     the doxorubicin transporter of Streptomyces peucetius. Gandlur
     Suvarna M; Wei Ling; Levine Jeoffery; Russell Jack; Kaur Parjit.
     (Department of Biology, Georgia State University, Atlanta, Georgia 30303,
     USA. ) Journal of biological chemistry, (2004 Jun 25) 279 (26) 27799-806.
     Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States.
     Language: English.
AB   Daunorubicin and doxorubicin, two commonly used anticancer agents, are
     produced by the soil bacterium Streptomyces peucetius.  Self-resistance to
     these antibiotics in S. peucetius is conferred by the drrAB locus that
     codes for two proteins, DrrA and DrrB.  DrrA is an
     ATP-binding protein.  It belongs to the ABC family of transporters
     and shares sequence and functional similarities with P-glycoprotein of
     cancer cells.  DrrB is an integral membrane protein that might function as
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a **transporter** for the efflux of daunorubicin and doxorubicin. Together, **DrrA** and DrrB are believed to form an ATP-driven pump for the efflux of these drugs. The drrAB locus has been cloned, and the two proteins have been expressed in a functional form in Escherichia coli. A topological analysis of the DrrB protein was performed using gene fusion methodology. Random and site-directed fusions of the drrB gene to lacZ, phoA, or gfp reporter genes were created. Based on the fusion data, a topological model of the DrrB protein is proposed in which the protein has eight membrane-spanning domains with both the N terminus and the C terminus in the cytoplasm.

L53 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2002:486237 Document No. 137:59285 Screening for regulators of ATP-binding cassette **transporters** and their use in diagnosis and treatment of cancer and bacterial, fungal and protozoal infections. (Universiteit Gent, Belg.). Eur. Pat. Appl. EP 1217066 A1 20020626, 290 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR. (English). CODEN: EPXXDW. APPLICATION: EP 2000-870316 20001221.

AB The invention relates to ATP-Binding Cassette (ABC) **transporters** and to mols. selectively modulating the activity of said ABC **transporters.** ABC **transporters** are heterodimeric proteins comprising 2 nucleotide binding domains. They comprise Walker Box A and B motifs and a D loop domain that is involved in dimerization. Mols. and compds. which selectively modulate the activity of specific ABC **transporters** by interfering with the dimerization interface are provided. The invention also relates to mols., compds. and compns. for preventing, treating or alleviating cancer or diseases related to bacterial, fungal or protozoal infections.

L53 ANSWER 3 OF 10 MEDLINE on STN DUPLICATE 2
2002479805. PubMed ID: 12057006. Overexpression and functional characterization of an ABC (ATP-binding cassette) **transporter** encoded by the genes **drrA** and drrB of Mycobacterium tuberculosis. Choudhuri Baisakhee Saha; Bhakta Sanjib; Barik Rajib; Basu Joyoti; Kundu Manikuntala; Chakrabarti Parul. (Department of Chemistry, Bose Institute, 93/1 Acharya Prafulla Chandra Road, Calcutta 700 009, India.) Biochemical journal, (2002 Oct 1) 367 (Pt 1) 279-85. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: England: United Kingdom. Language: English.

AB The genes encoding ATP-binding cassette (ABC) **transporters** occupy 2.5% of the genome of Mycobacterium tuberculosis. However, none of these putative ABC **transporters** has been characterized so far. We describe the development of expression systems for simultaneous expression of the ATP-binding protein **DrrA** and the membrane integral protein DrrB which together behave as a functional doxorubicin efflux pump. Doxorubicin uptake in Escherichia coli or Mycobacterium smegmatis expressing DrrAB was inhibited by reserpine, an inhibitor of ABC **transporters.** The localization of **DrrA** to the membrane depended on the simultaneous expression of DrrB. ATP binding was positively regulated by doxorubicin and daunorubicin. At the same time, DrrB appeared to be sensitive to proteolysis when expressed alone in the absence of **DrrA.** Simultaneous expression of the two polypeptides was essential to obtain a functional doxorubicin efflux pump. Expression of DrrAB in E. coli conferred 8-fold increased resistance to ethidium bromide, a cationic compound. 2',7'-bis-(2-Carboxyethyl)-5(6)-carboxyfluorescein (BCECF), a neutral compound, also behaved as a substrate of the reconstituted efflux pump. When expressed in M. smegmatis, DrrAB conferred resistance to a number of clinically relevant, structurally unrelated antibiotics. The resistant phenotype could be reversed by verapamil and reserpine, two potent inhibitors of ABC **transporters.**

L53 ANSWER 4 OF 10 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:597305 Document No.: PREV200200597305. Characterization of interaction
between the components of the DrrAB antibiotic efflux pump in Streptomyces
peucetius. Gandlur, S. M. [Reprint author]; Kaur, P. [Reprint author].
Georgia State University, Atlanta, GA, USA. Abstracts of the General
Meeting of the American Society for Microbiology, (2002) Vol. 102, pp.
273. print.
Meeting Info.: 102nd General Meeting of the American Society for
Microbiology. Salt Lake City, UT, USA. May 19-23, 2002. American Society
for Microbiology.
ISSN: 1060-2011. Language: English.

AB Streptomyces peucetius produces clinically important antitumor antibiotics, doxorubicin and daunorubicin, and is self-resistant to the inhibitory action of these two antibiotics. It has been hypothesized that an antibiotic efflux pump formed by two proteins, **DrrA** and DrrB, results in resistance to these antibiotics in this organism. The DrrAB system shows sequence and functional similarity to the multi-drug resistance pump p-glycoprotein in cancer cells. P-glycoprotein confers resistance to a wide variety of unrelated drugs, including doxorubicin and daunorubicin, by an ATP dependent efflux mechanism. Both DrrAB and p-glycoprotein belong to the ABC family of **transporters.** Unlike p-glycoprotein, which is one protein of about 170 kDa, the Drr system consists of two proteins, **DrrA** and DrrB. Both the proteins have been expressed and studied in E. coli. **DrrA**, the ABC component of the pump, binds ATP in a doxorubicin-dependent manner. DrrB is a hydrophobic protein that is localized to the inner membrane in E. coli. The two proteins show strong interdependence on each other for stability and function, which suggests interaction and possible complex formation between the two proteins. The present study looks at possible interaction sites between the two proteins by cysteine cross-linking studies.

L53 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in
cancer and infectious lesions using immunoconjugates. Goldenberg, David
M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316,
40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English).
CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US
1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L53 ANSWER 6 OF 10 MEDLINE on STN
1998316372. PubMed ID: 9651400. Biochemical coupling between the
DrrA and DrrB proteins of the doxorubicin efflux pump of
Streptomyces peucetius. Kaur P; Russell J. (Department of Biology, Georgia
State University, Atlanta, Georgia 30303, USA.. bioppk@panther.gsu.edu) .
Journal of biological chemistry, (1998 Jul 10) 273 (28) 17933-9. Journal
code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language:
English.

AB The drrAB operon of Streptomyces peucetius encodes for resistance to the antibiotics doxorubicin and daunorubicin. Subcloning of the drrAB genes in Escherichia coli has previously been shown to result in expression of **DrrA** and DrrB proteins and resistance to doxorubicin in a sensitive strain of E. coli. **DrrA**, a peripheral membrane protein, binds ATP in a UV-catalyzed reaction in a doxorubicin-dependent manner; DrrB, a hydrophobic protein, is localized to the inner membrane of

E. coli (Kaur, P. (1997) J. Bacteriol. 179, 569-575). The present study provides evidence that DrrB, the membrane component of the complex, is stably maintained in the cell only if **DrrA** is present. Furthermore, it was found that the catalytic component **DrrA** is in an active conformation only when it is in a complex with DrrB. In a subclone containing the drrB gene by itself, no DrrB protein could be detected, although a translational fusion of the first 15 amino acids of DrrB to beta-galactosidase indicated that DrrB is translated in the absence of **DrrA.** Upon co-transformation with a plasmid containing the **drrA** gene in trans, DrrB could again be detected in these cells. UV cross-linking studies with [alpha-32P]ATP showed that only the membrane-bound form of **DrrA** in cells containing both **DrrA** and DrrB was in a conformation competent to bind ATP. Chemical cross-linking studies also provided direct evidence for interaction between the two proteins. Based on these analyses, a model for interaction between **DrrA** and DrrB proteins is presented.

L53 ANSWER 7 OF 10 MEDLINE on STN DUPLICATE 3
97158646. PubMed ID: 9006006. Expression and characterization of **DrrA** and DrrB proteins of Streptomyces peucetius in Escherichia coli: **DrrA** is an ATP binding protein. Kaur P. (Department of Biology, Georgia State University, Atlanta 30303, USA.. bioppk@gsusgi2.gsu.edu) . Journal of bacteriology, (1997 Feb) 179 (3) 569-75. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB Streptomyces peucetius, a microorganism that produces the anticancer drugs doxorubicin and daunorubicin, is itself resistant to the action of these drugs. The genes conferring resistance to doxorubicin and daunorubicin in S. peucetius have been sequenced (P. G. Guilfoile and R. Hutchinson, Proc. Natl. Acad. Sci. USA 88:8553-8557, 1991). Two open reading frames, **drrA** and drrB, were proposed to encode for an ABC (ATP-binding cassette) type of permease that carries out export of the antibiotics in an ATP-dependent manner. This article reports subcloning of the **drrA** and drrB genes into Escherichia coli expression vectors and characterization of their gene products. Upon induction from the lac promoter, a 36-kDa **DrrA** protein could be identified on Coomassie blue-stained gels. The DrrB protein was identified by use of a polyclonal antiserum generated against a synthetic peptide corresponding to a portion of the DrrB protein. Together, the **DrrA** and DrrB proteins conferred resistance to doxorubicin in E. coli. The DrrB protein was localized to the cell membrane. The **DrrA** protein bound ATP or GTP in a Mg2+-dependent fashion. ATP binding was enhanced on addition of doxorubicin or daunorubicin.

L53 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US9491 19950801. PRIORITY: US 1994-286430 19940805.

AB Polyspecific immunoconjugates and antibody composites that bind a multidrug **transporter** protein and an antigen associated with a tumor or infectious agent are used to overcom the multidrug resistant phenotype. These immunoconjugates and composites also can be used diagnostically to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents. In example, polyspecific immunoconjugates containing G-CSF, 90Yttrium, doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen bispecific antibody were prepared for colon cancer treatment. 99mTc- and

111Indium-labeled polyspecific immunoconjugate targeted to multidrug resistant Pseudomonas aeruginosa were also prepared

L53 ANSWER 9 OF 10 MEDLINE on STN
94254733. PubMed ID: 8196549. An ABC-**transporter** from Streptomyces longisporoflavus confers resistance to the polyether-ionophore antibiotic tetronasin. Linton K J; Cooper H N; Hunter I S; Leadlay P F. (Robertson Institute of Biotechnology, Department of Genetics, University of Glasgow, UK.) Molecular microbiology, (1994 Feb) 11 (4) 777-85. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Streptomyces longisporoflavus produces the polyketide-polyether antibiotic, tetronasin, which acts as an ionophore and depolarizes the membrane of bacteria sensitive to the drug. A genomic library of S. longisporoflavus DNA was cloned in Streptomyces lividans and screened to identify tetronasin-resistance determinants. The inclusion of 0.2M NaCl in the growth medium with tetronasin markedly improved the sensitivity of the screen. Two different resistance determinants, designated tnrB (ptetR51) and tnrA (ptetR11) respectively, were identified. The determinant tnrB (ptetR51) but not tnrA (ptetR11), also conferred resistance to tetronasin when cloned into Streptomyces albus. The tnrB determinant was further localized, by subcloning, to a 2.8 kb KpnI fragment. DNA sequence analysis of this insert revealed one incomplete and two complete open reading frames (ORFs 1, 2 and 3). The deduced sequence of the gene product of ORF2 (TnrB2) revealed significant similarity to the ATP-binding domains of the ABC (ATP binding cassette) superfamily of transport-related proteins. The adjacent gene, ORF3, is translationally coupled to ORF2 and would encode a hydrophobic protein (TnrB3) with six transmembrane helices which probably constitutes the integral membrane component of the **transporter.** The mechanism of tetronasin resistance mediated by tnrB is probably an ATP-dependent efflux system.

L53 ANSWER 10 OF 10 MEDLINE on STN
93316854. PubMed ID: 8326867. Streptomyces antibioticus contains at least three oleandomycin-resistance determinants, one of which shows similarity with proteins of the ABC-**transporter** superfamily. Rodriguez A M; Olano C; Vilches C; Mendez C; Salas J A. (Departamento de Biologia Funcional, Universidad de Oviedo, Spain.) Molecular microbiology, (1993 May) 8 (3) 571-82. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Three different DNA fragments of an oleandomycin producer, Streptomyces antibioticus, conferring oleandomycin resistance were cloned in plasmid pIJ702 and expressed in Streptomyces lividans and in Streptomyces albus. These oleandomycin resistance determinants were designated as oleA (pOR400), oleB (pOR501) and oleC (pOR800). oleA and oleC are closely linked in the chromosome as they were both obtained together in two cosmid clones that were isolated from a genomic library. Sequencing of the oleC resistance determinant revealed four complete open reading frames (ORFs) and the C-terminal end of a fifth. The functions of orf1 and orf2 are unknown since they did not show significant similarity with other sequences in the data bases. The orf3 gene product has similarity with some proteins involved in iron and vitamin B12 uptake in bacteria. The orf4 gene product had a hydrophilic profile and showed important similarity with proteins containing typical ATP-binding domains characteristic of the ABC-**transporter** superfamily and involved in membrane transport and, particularly, with several genes conferring resistance to various macrolide antibiotics and anticancer drugs. The last gene, orf5, is translationally coupled to orf4 and codes for a hydrophobic polypeptide containing several transmembrane domains characteristic of integral membrane proteins. Subcloning and deletion experiments limited the resistance determinant to a 0.9 kb PstI-SphI fragment and only orf4 is included in this fragment. These results suggest that resistance to oleandomycin conferred by oleC (orf4) is probably due to an efflux transport system of the ABC-**transporter**

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     superfamily.


=> s l11 and "DrrB"
L54          18 L11 AND "DRRB"

=> dup remove l54
PROCESSING COMPLETED FOR L54
L55           9 DUP REMOVE L54 (9 DUPLICATES REMOVED)

=> d l55 1-9 cbib abs

L55  ANSWER 1 OF 9     MEDLINE on STN                    DUPLICATE 1
2004305775.     PubMed ID: 15090538.   Membrane topology of the DrrB
     protein of the doxorubicin transporter of Streptomyces
     peucetius. Gandlur Suvarna M; Wei Ling; Levine Jeoffery; Russell Jack;
     Kaur Parjit. (Department of Biology, Georgia State University, Atlanta,
     Georgia 30303, USA. ) Journal of biological chemistry, (2004 Jun 25) 279
     (26) 27799-806.  Journal code: 2985121R. ISSN: 0021-9258. Pub. country:
     United States. Language: English.
AB   Daunorubicin and doxorubicin, two commonly used anticancer agents, are
     produced by the soil bacterium Streptomyces peucetius.  Self-resistance to
     these antibiotics in S. peucetius is conferred by the drrAB locus that
     codes for two proteins, DrrA and DrrB.  DrrA is an ATP-binding
     protein.  It belongs to the ABC family of transporters and
     shares sequence and functional similarities with P-glycoprotein of cancer
     cells.  DrrB is an integral membrane protein that might function
     as a transporter for the efflux of daunorubicin and doxorubicin.
     Together, DrrA and DrrB are believed to form an ATP-driven pump
     for the efflux of these drugs.  The drrAB locus has been cloned, and the
     two proteins have been expressed in a functional form in Escherichia coli.
     A topological analysis of the DrrB protein was performed using
     gene fusion methodology.  Random and site-directed fusions of the
     drrB gene to lacZ, phoA, or gfp reporter genes were created.
     Based on the fusion data, a topological model of the DrrB
     protein is proposed in which the protein has eight membrane-spanning
     domains with both the N terminus and the C terminus in the cytoplasm.

L55  ANSWER 2 OF 9  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation.  on
     STN
2003:146986  The Genuine Article (R) Number: 640GY. The structure and function
     of drug pumps: an update.  McKeegan K S; Borges-Walmsley M I; Walmsley A R
     (Reprint). Univ Durham, Wolfson Res Inst, Ctr Infect Dis, Queens Campus,
     Durham TS17 6BH, England (Reprint); Univ Durham, Wolfson Res Inst, Ctr
     Infect Dis, Durham TS17 6BH, England. TRENDS IN MICROBIOLOGY (JAN 2003)
     Vol. 11, No. 1, pp. 21-29. Publisher: ELSEVIER SCIENCE LONDON. 84
     THEOBALDS RD, LONDON WC1X 8RR, ENGLAND. ISSN: 0966-842X. Pub. country:
     England. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB      Our understanding of the exact mechanisms used by the transmembrane
     protein pumps that confer cellular resistance to cytotoxic drugs has
     improved enormously with the recent determination of the structures of
     three Escherichia coli transporters, two belonging to the
     ATP-binding cassette (ABC) superfamily and one to the resistance-
     nodulation-cell division (RND) family. Although these studies do not
     provide an insight into how drug pumps can recognize several structurally
     unrelated drugs, important advances have been also made in this area.
     Information on the molecular basis of multidrug recognition has been
     provided by determining the structure of transcriptional regulators that
     can bind, often structurally unrelated, cytotoxic drugs and control the
     expression of drug pumps.

L55  ANSWER 3 OF 9     MEDLINE on STN                    DUPLICATE 2
2002479805.     PubMed ID: 12057006.   Overexpression and functional
     characterization of an ABC (ATP-binding cassette) transporter
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encoded by the genes drrA and **drrB** of Mycobacterium tuberculosis. Choudhuri Baisakhee Saha; Bhakta Sanjib; Barik Rajib; Basu Joyoti; Kundu Manikuntala; Chakrabarti Parul. (Department of Chemistry, Bose Institute, 93/1 Acharya Prafulla Chandra Road, Calcutta 700 009, India.) Biochemical journal, (2002 Oct 1) 367 (Pt 1) 279-85. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: England: United Kingdom. Language: English.

AB The genes encoding ATP-binding cassette (ABC) **transporters** occupy 2.5% of the genome of Mycobacterium tuberculosis. However, none of these putative ABC **transporters** has been characterized so far. We describe the development of expression systems for simultaneous expression of the ATP-binding protein DrrA and the membrane integral protein **DrrB** which together behave as a functional doxorubicin efflux pump. Doxorubicin uptake in Escherichia coli or Mycobacterium smegmatis expressing DrrAB was inhibited by reserpine, an inhibitor of ABC **transporters.** The localization of DrrA to the membrane depended on the simultaneous expression of **DrrB.** ATP binding was positively regulated by doxorubicin and daunorubicin. At the same time, **DrrB** appeared to be sensitive to proteolysis when expressed alone in the absence of DrrA. Simultaneous expression of the two polypeptides was essential to obtain a functional doxorubicin efflux pump. Expression of DrrAB in E. coli conferred 8-fold increased resistance to ethidium bromide, a cationic compound. 2',7'-bis-(2-Carboxyethyl)-5(6)-carboxyfluorescein (BCECF), a neutral compound, also behaved as a substrate of the reconstituted efflux pump. When expressed in M. smegmatis, DrrAB conferred resistance to a number of clinically relevant, structurally unrelated antibiotics. The resistant phenotype could be reversed by verapamil and reserpine, two potent inhibitors of ABC **transporters.**

L55 ANSWER 4 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2002:597305 Document No.: PREV200200597305. Characterization of interaction between the components of the DrrAB antibiotic efflux pump in Streptomyces peucetius. Gandlur, S. M. [Reprint author]; Kaur, P. [Reprint author]. Georgia State University, Atlanta, GA, USA. Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 273. print.

Meeting Info.: 102nd General Meeting of the American Society for Microbiology. Salt Lake City, UT, USA. May 19-23, 2002. American Society for Microbiology.

ISSN: 1060-2011. Language: English.

AB Streptomyces peucetius produces clinically important antitumor antibiotics, doxorubicin and daunorubicin, and is self-resistant to the inhibitory action of these two antibiotics. It has been hypothesized that an antibiotic efflux pump formed by two proteins, DrrA and **DrrB,** results in resistance to these antibiotics in this organism. The DrrAB system shows sequence and functional similarity to the multi-drug resistance pump p-glycoprotein in cancer cells. P-glycoprotein confers resistance to a wide variety of unrelated drugs, including doxorubicin and daunorubicin, by an ATP dependent efflux mechanism. Both DrrAB and p-glycoprotein belong to the ABC family of **transporters.** Unlike p-glycoprotein, which is one protein of about 170 kDa, the Drr system consists of two proteins, DrrA and **DrrB.** Both the proteins have been expressed and studied in E. coli. DrrA, the ABC component of the pump, binds ATP in a doxorubicin-dependent manner. **DrrB** is a hydrophobic protein that is localized to the inner membrane in E. coli. The two proteins show strong interdependence on each other for stability and function, which suggests interaction and possible complex formation between the two proteins. The present study looks at possible interaction sites between the two proteins by cysteine cross-linking studies.

L55 ANSWER 5 OF 9 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2001:473025 The Genuine Article (R) Number: 438MW. The role of ABC **transporters** in antibiotic-producing organisms: drug secretion and resistance mechanisms. Mendez C; Salas J A (Reprint). Univ Oviedo, Dept Biol Func, E-33006 Oviedo, Spain (Reprint); Univ Oviedo, Inst Univ Oncol Principado Asturias, E-33006 Oviedo, Spain. RESEARCH IN MICROBIOLOGY (APR-MAY 2001) Vol. 152, No. 3-4, pp. 341-350. Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER. 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE. ISSN: 0923-2508. Pub. country: Spain. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Knowledge about biosynthetic gene clusters from antibiotic-producing actinomycetes is continuously increasing and the presence of an ABC **transporter** system is a fairly general phenomenon in most of these clusters. These **transporters** are involved in the secretion of the antibiotic through the cell membrane and also contribute to self resistance to the produced antibiotic. (C) 2001 Editions scientifiques et medicales Elsevier SAS.

L55 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN

2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

AB Immunoconjugates of a diagnostic agent and an antibody component that binds an epitope of a multidrug **transporter** protein are disclosed. Thesse immunoconjugates are used in in vivo diagnostic methods to determine whether the failure of traditional chemotherapy is due to the presence of multidrug resistant tumor cells, multidrug resistant HIV-infected cells or multidrug resistant infectious agents.

L55 ANSWER 7 OF 9 MEDLINE on STN

1998316372. PubMed ID: 9651400. Biochemical coupling between the DrrA and **DrrB** proteins of the doxorubicin efflux pump of Streptomyces peucetius. Kaur P; Russell J. (Department of Biology, Georgia State University, Atlanta, Georgia 30303, USA.. bioppk@panther.gsu.edu) . Journal of biological chemistry, (1998 Jul 10) 273 (28) 17933-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The drrAB operon of Streptomyces peucetius encodes for resistance to the antibiotics doxorubicin and daunorubicin. Subcloning of the drrAB genes in Escherichia coli has previously been shown to result in expression of DrrA and **DrrB** proteins and resistance to doxorubicin in a sensitive strain of E. coli. DrrA, a peripheral membrane protein, binds ATP in a UV-catalyzed reaction in a doxorubicin-dependent manner; **DrrB**, a hydrophobic protein, is localized to the inner membrane of E. coli (Kaur, P. (1997) J. Bacteriol. 179, 569-575). The present study provides evidence that **DrrB**, the membrane component of the complex, is stably maintained in the cell only if DrrA is present. Furthermore, it was found that the catalytic component DrrA is in an active conformation only when it is in a complex with **DrrB**. In a subclone containing the **drrB** gene by itself, no **DrrB** protein could be detected, although a translational fusion of the first 15 amino acids of **DrrB** to beta-galactosidase indicated that **DrrB** is translated in the absence of DrrA. Upon co-transformation with a plasmid containing the drrA gene in trans, **DrrB** could again be detected in these cells. UV cross-linking studies with [alpha-32P]ATP showed that only the membrane-bound form of DrrA in cells containing both DrrA and **DrrB** was in a conformation competent to bind ATP. Chemical cross-linking studies also provided direct evidence for interaction between the two proteins. Based on these analyses, a

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     model for interaction between DrrA and DrrB proteins is
     presented.

L55  ANSWER 8 OF 9     MEDLINE on STN                       DUPLICATE 3
97158646.     PubMed ID: 9006006.   Expression and characterization of DrrA and
     DrrB proteins of Streptomyces peucetius in Escherichia coli: DrrA
     is an ATP binding protein. Kaur P. (Department of Biology, Georgia State
     University, Atlanta 30303, USA.. bioppk@gsusgi2.gsu.edu) . Journal of
     bacteriology, (1997 Feb) 179 (3) 569-75.  Journal code: 2985120R. ISSN:
     0021-9193. Pub. country: United States. Language: English.
AB   Streptomyces peucetius, a microorganism that produces the anticancer drugs
     doxorubicin and daunorubicin, is itself resistant to the action of these
     drugs.  The genes conferring resistance to doxorubicin and daunorubicin in
     S. peucetius have been sequenced (P.  G.  Guilfoile and R.  Hutchinson,
     Proc.  Natl.  Acad.  Sci.  USA 88:8553-8557, 1991).  Two open reading
     frames, drrA and drrB, were proposed to encode for an ABC
     (ATP-binding cassette) type of permease that carries out export of the
     antibiotics in an ATP-dependent manner.  This article reports subcloning
     of the drrA and drrB genes into Escherichia coli expression
     vectors and characterization of their gene products.  Upon induction from
     the lac promoter, a 36-kDa DrrA protein could be identified on Coomassie
     blue-stained gels.  The DrrB protein was identified by use of a
     polyclonal antiserum generated against a synthetic peptide corresponding
     to a portion of the DrrB protein.  Together, the DrrA and
     DrrB proteins conferred resistance to doxorubicin in E. coli.  The
     DrrB protein was localized to the cell membrane.  The DrrA protein
     bound ATP or GTP in a Mg2+-dependent fashion.  ATP binding was enhanced on
     addition of doxorubicin or daunorubicin.

L55  ANSWER 9 OF 9  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658    Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared


=> s l11 and "TlrC"
L56          3 L11 AND "TLRC"

=> dup remove l56
PROCESSING COMPLETED FOR L56
L57             3 DUP REMOVE L56 (0 DUPLICATES REMOVED)

=> d l57 1-3 cbib abs

L57  ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017    Document No. 132:219218  Diagnosis of multidrug resistance in
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     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
AB   Immunoconjugates of a diagnostic agent and an antibody component that
     binds an epitope of a multidrug transporter protein are
     disclosed.  Thesse immunoconjugates are used in in vivo diagnostic methods
     to determine whether the failure of traditional chemotherapy is due to the
     presence of multidrug resistant tumor cells, multidrug resistant
     HIV-infected cells or multidrug resistant infectious agents.

L57  ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658   Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared

L57  ANSWER 3 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
1993:249954   Document No. 118:249954  Sequence similarity between
     macrolide-resistance determinants and ATP-binding transport proteins.
     Schoner, Brigitte; Geistlich, Martin; Rosteck, Paul, Jr.; Rao, R.
     Nagaraja; Seno, Eugene; Reynolds, Patricia; Cox, Karen; Burgett, Stanley;
     Hershberger, Charles (Lilly Res. Lab., Indianapolis, IN, 46285, USA).
     Gene, 115(1-2), 93-6 (English) 1992.  CODEN: GENED6.  ISSN: 0378-1119.
AB   The three macrolide-resistance-encoding genes, tlrC from
     Streptomyces fradiae, srmB from S. ambofaciens, and carA from S.
     thermotolerans, encode proteins that possess significant sequence
     similarity to ATP-dependent transport proteins.  The N-terminal and
     C-terminal halves of these proteins are very similar to each other and
     contain highly conserved regions that resemble ATP-binding domains
     typically present within the superfamily of ATP-dependent transport
     proteins.  These observations suggest that the mechanism by which these
     genes confer resistance to macrolides is due to export of the antibiotics,
     a process that is driven by energy derived from ATP hydrolysis.


=> s l11 and "Bmr"
L58         144 L11 AND "BMR"

=> sup remove l58
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SUP IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> dup remove l58
PROCESSING COMPLETED FOR L58
L59           47 DUP REMOVE L58 (97 DUPLICATES REMOVED)

=> d l59 1-47 cbib

L59  ANSWER 1 OF 47    MEDLINE on STN                    DUPLICATE 1
2004224973.    PubMed ID: 14985361.   The structural mechanism for
     transcription activation by MerR family member multidrug
     transporter activation, N terminus. Newberry Kate J; Brennan
     Richard G. (Department of Biochemistry and Molecular Biology, Oregon
     Health & Science University, Portland, OR 97239-3098, USA. ) Journal of
     biological chemistry, (2004 May 7) 279 (19) 20356-62.  Journal code:
     2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

L59  ANSWER 2 OF 47    MEDLINE on STN
2004336912.    PubMed ID: 15182191.   Characterization of YvcC (BmrA), a
     multidrug ABC transporter constitutively expressed in Bacillus
     subtilis. Steinfels Emmanuelle; Orelle Cedric; Fantino Jean-Raphael;
     Dalmas Olivier; Rigaud Jean-Louis; Denizot Francois; Di Pietro Attilio;
     Jault Jean-Michel. (Institut de Biologie et Chimie des Proteines, UMR 5086
     CNRS-UCBL et IFR 128, 7 passage du Vercors, 69367 Lyon Cedex 07, France. )
     Biochemistry, (2004 Jun 15) 43 (23) 7491-502.  Journal code: 0370623.
     ISSN: 0006-2960. Pub. country: United States. Language: English.

L59  ANSWER 3 OF 47  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation.  on
     STN
2004:761333  The Genuine Article (R) Number: 847DE. Effect of substrate
     exposure and other growth condition manipulations on norA expression.
     Kaatz G W (Reprint); Seo S M. Wayne State Univ, Sch Med, John D Dingell
     Dept Vet Affairs Med Ctr, B4333 John D Dingell VA Med Ctr, 4646 John R,
     Detroit, MI 48201 USA (Reprint); Wayne State Univ, Sch Med, John D Dingell
     Dept Vet Affairs Med Ctr, Detroit, MI 48201 USA; Wayne State Univ, Sch
     Med, Dept Internal Med, Div Infect Dis, Detroit, MI 48201 USA. JOURNAL OF
     ANTIMICROBIAL CHEMOTHERAPY (AUG 2004) Vol. 54, No. 2, pp. 364-369.
     Publisher: OXFORD UNIV PRESS. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
     ISSN: 0305-7453. Pub. country: USA. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

L59  ANSWER 4 OF 47  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation.  on
     STN                                                 DUPLICATE 2
2004:156827  Document No.: PREV200400150555. Discrimination of Bacillus
     subtilis natto "strain KA-145" using insersion sequence, IS4Bsu1. Inagaki,
     Shyuichiro [Reprint Author]; Domon, Eiji; Saito, Akira; Akutagawa,
     Hiroshi. Marumiya Co., Ltd., Uchida 2211, Kikusui, Tamana, Kumamoto,
     865-0104, Japan. em1068@marumiya-g.co.jp. Nippon Shokuhin Kagaku Kogaku
     Kaishi, (2003) Vol. 50, No. 10, pp. 483-487. print.
     ISSN: 1341-027X (ISSN print). Language: Japanese.

L59  ANSWER 5 OF 47  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation.  on
     STN
2003:129727  The Genuine Article (R) Number: 641RW. Functional cloning and
     expression of emeA, and characterization of EmeA, a multidrug efflux pump
     from Enterococcus faecalis.  Lee E W; Chen J; Huda M N; Kuroda T;
     Mizushima T; Tsuchiya T (Reprint). Okayama Univ, Fac Pharmaceut Sci, Dept
     Microbiol, Okayama 7008530, Japan (Reprint). BIOLOGICAL & PHARMACEUTICAL
     BULLETIN (FEB 2003) Vol. 26, No. 2, pp. 266-270. Publisher: PHARMACEUTICAL
     SOC JAPAN. 2-12-15-201 SHIBUYA, SHIBUYA-KU, TOKYO, 150, JAPAN. ISSN:
     0918-6158. Pub. country: Japan. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
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L59  ANSWER 6 OF 47  CAPLUS  COPYRIGHT 2005 ACS on STN
2003:647295   Document No. 139:286850  Multidrug resistance: Problem solved?.
     Neyfakh, A. A. (Center for Pharmaceutical Biotechnology (MIC 870),
     University of Illinois, Chicago, IL, 60607, USA).  Biologicheskie
     Membrany, 20(3), 206-212 (Russian) 2003.  CODEN: BIMEE9.  ISSN: 0233-4755.
     Publisher: Nauka.

L59  ANSWER 7 OF 47  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation.  on
     STN
2002:278285  Document No.: PREV200200278285. The ostensible paradox of
     multidrug recognition. Neyfakh, Alex A. [Reprint author]. Center for
     Pharmaceutical Biotechnology, University of Illinois, 900 S. Ashland Ave.,
     Chicago, IL, 60607, USA. neyfakh@uic.edu. Paulsen, Ian T. [Editor]; Lewis,
     Kim [Editor]. (2002) pp. 21-30. JMMB Symposium Series. Microbial multidrug
     efflux. print. Publisher: Horizon Scientific Press, 32 Hewitts Lane,
     Wymondham, Norfolk, NR18 0JA, UK. Series: JMMB Symposium Series.
     ISBN: 1-898486-33-6 (cloth). Language: English.

L59  ANSWER 8 OF 47  BIOSIS  COPYRIGHT (c) 2005 The Thomson Corporation.  on
     STN
2002:278283  Document No.: PREV200200278283. Microbial multidrug efflux:
     Introduction. Paulsen, Ian T. [Reprint author]; Lewis, Kim. The Institute
     for Genomic Research, 9712 Medical Center Drive, Rockville, MD, 20850,
     USA. ipaulsen@tigr.org. Paulsen, Ian T. [Editor]; Lewis, Kim [Editor].
     (2002) pp. 1-4. JMMB Symposium Series. Microbial multidrug efflux. print.
     Publisher: Horizon Scientific Press, 32 Hewitts Lane, Wymondham, Norfolk,
     NR18 0JA, UK. Series: JMMB Symposium Series.
     ISBN: 1-898486-33-6 (cloth). Language: English.

L59  ANSWER 9 OF 47     MEDLINE on STN                    DUPLICATE 3
2002682602.    PubMed ID: 12443939.   Prolonged longevity in naked mole-rats:
     age-related changes in metabolism, body composition and gastrointestinal
     function. O'Connor Timothy P; Lee Angela; Jarvis Jennifer U M; Buffenstein
     Rochelle. (Department of Biology, City College of City University of New
     York, 138th Street and Convent Avenue, New York, NY 10031, USA. )
     Comparative biochemistry and physiology. Part A, Molecular & integrative
     physiology, (2002 Nov) 133 (3) 835-42.  Journal code: 9806096. ISSN:
     1095-6433. Pub. country: United States. Language: English.

L59  ANSWER 10 OF 47  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation.
     on STN
2003:11714   The Genuine Article (R) Number: 622VY. Identification and
     characterization of a novel efflux-related multidrug resistance phenotype
     in Staphylococcus aureus.  Kaatz G W (Reprint); Moudgal V V; Seo S M.
     Wayne State Univ, Sch Med, John D Dingell Dept Vet Affairs Med Ctr,
     Detroit, MI 48201 USA (Reprint); Wayne State Univ, Sch Med, Dept Internal
     Med, Div Infect Dis, Detroit, MI 48201 USA. JOURNAL OF ANTIMICROBIAL
     CHEMOTHERAPY (DEC 2002) Vol. 50, No. 6, pp. 833-838. Publisher: OXFORD
     UNIV PRESS. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND. ISSN: 0305-7453.
     Pub. country: USA. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

L59  ANSWER 11 OF 47  CAPLUS  COPYRIGHT 2005 ACS on STN
2003:433942   Document No. 139:112465  Cloning and selective toxicant-induced
     expression of BMR1 and BMR3, novel ABC transporter genes in
     Botrytis cinerea.  Makizumi, Yoshiyuki; Takeda, Shin-ichi; Matsuzaki,
     Yuichi; Nakaune, Ryoji; Hamamoto, Hiroshi; Akutsu, Katsumi; Hibi, Tadaaki
     (Department of Agricultural and Environmental Biology, The University of
     Tokyo, Tokyo, 113-8657, Japan).  Journal of General Plant Pathology,
     68(4), 338-341 (English) 2002.  CODEN: JGPPBQ.  ISSN: 1345-2630.
     Publisher: Phytopathological Society of Japan.

L59  ANSWER 12 OF 47  CAPLUS  COPYRIGHT 2005 ACS on STN
2001:507951   Document No. 135:87148  Metal ion binding site-based method of
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identifying ligands of biological target molecules for drug discovery. Elling, Christian E.; Gerlach, Lars Ole; Holst Lange, Birgitte; Pedersen, Jan Torleif; Schwartz, Thue W. (7TM Pharma, Den.). PCT Int. Appl. WO 2001050127 A2 20010712, 114 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-EP13389 20001229. PRIORITY: DK 1999-1879 19991230; DK 1999-1880 19991230; US 2000-PV175401 20000111; US 2000-PV175994 20000111; DK 2000-705 20000428; US 2000-PV202990 20000509.

L59 ANSWER 13 OF 47 MEDLINE on STN DUPLICATE 4
2001118754. PubMed ID: 11201751. Crystal structure of the transcription activator BmrR bound to DNA and a drug. Heldwein E E; Brennan R G. (Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland 97201-3098, USA.) Nature, (2001 Jan 18) 409 (6818) 378-82. Journal code: 0410462. ISSN: 0028-0836. Pub. country: England: United Kingdom. Language: English.

L59 ANSWER 14 OF 47 MEDLINE on STN DUPLICATE 5
2001483518. PubMed ID: 11321567. The ostensible paradox of multidrug recognition. Neyfakh A A. (Center for Pharmaceutical Biotechnology, University of Illinois, Chicago 60607, USA.. neyfakh@uic.edu) . Journal of molecular microbiology and biotechnology, (2001 Apr) 3 (2) 151-4. Ref: 23. Journal code: 100892561. ISSN: 1464-1801. Pub. country: England: United Kingdom. Language: English.

L59 ANSWER 15 OF 47 CAPLUS COPYRIGHT 2005 ACS on STN
2000:383932 Document No. 133:26839 Inhibitors of multidrug **transporters** for enhancing the action of antibacterial and antifungal agents. Markham, Penelope N.; Mulhearn, Debbie C.; Neyfakh, Alexander A.; Crich, David; Jaber, Mohamad-Rami; Johnson, Michael E. (Influx, Inc., USA). PCT Int. Appl. WO 2000032196 A2 20000608, 119 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US28732 19991202. PRIORITY: US 1998-PV110841 19981204.

L59 ANSWER 16 OF 47 CAPLUS COPYRIGHT 2005 ACS on STN
2000:176017 Document No. 132:219218 Diagnosis of multidrug resistance in cancer and infectious lesions using immunoconjugates. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 2000014537 A2 20000316, 40 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US20017 19990901. PRIORITY: US 1998-PV99304 19980904.

L59 ANSWER 17 OF 47 MEDLINE on STN DUPLICATE 6
2001114168. PubMed ID: 10961951. Recognition of multiple drugs by a single protein: a trivial solution of an old paradox. Vazquez-Laslop N; Zheleznova E E; Markham P N; Brennan R G; Neyfakh A A. (Center for

Pharmaceutical Biotechnology (M/C 870), University of Illinois, 900 South Ashland Avenue, Chicago, IL 60607, USA.) Biochemical Society transactions, (2000) 28 (4) 517-20. Ref: 18. Journal code: 7506897. ISSN: 0300-5127. Pub. country: ENGLAND: United Kingdom. Language: English.

L59 ANSWER 18 OF 47 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:156519 The Genuine Article (R) Number: 285ZA. A structure-based mechanism for drug binding by multidrug **transporters.**
Zheleznova E E (Reprint); Markham P; Edgar R; Bibi E; Neyfakh A A; Brennan R G. OREGON HLTH SCI UNIV, DEPT BIOCHEM & MOL BIOL, PORTLAND, OR 97201 (Reprint); UNIV ILLINOIS, CTR PHARMACEUT BIOTECHNOL, CHICAGO, IL; WEIZMANN INST SCI, DEPT BIOL CHEM, IL-76100 REHOVOT, ISRAEL. TRENDS IN BIOCHEMICAL SCIENCES (FEB 2000) Vol. 25, No. 2, pp. 39-43. Publisher: ELSEVIER SCIENCE LONDON. 84 THEOBALDS RD, LONDON WC1X 8RR, ENGLAND. ISSN: 0968-0004. Pub. country: USA; ISRAEL. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L59 ANSWER 19 OF 47 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
2000:36507 The Genuine Article (R) Number: 270YC. Mechanism of ligand recognition by BmrR, the multidrug-responding transcriptional regulator: Mutational analysis of the ligand-binding site. VazquezLaslop N; Markham P N; Neyfakh A A (Reprint). UNIV ILLINOIS, CTR PHARMACEUT BIOTECHNOL MC870, 900 S ASHLAND AVE, CHICAGO, IL 60607 (Reprint); UNIV ILLINOIS, CTR PHARMACEUT BIOTECHNOL MC870, CHICAGO, IL 60607. BIOCHEMISTRY (21 DEC 1999) Vol. 38, No. 51, pp. 16925-16931. Publisher: AMER CHEMICAL SOC. 1155 16TH ST, NW, WASHINGTON, DC 20036. ISSN: 0006-2960. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L59 ANSWER 20 OF 47 MEDLINE on STN DUPLICATE 7
1999217029. PubMed ID: 10200972. Mta, a global MerR-type regulator of the Bacillus subtilis multidrug-efflux **transporters.** Baranova N N; Danchin A; Neyfakh A A. (Center for Pharmaceutical Biotechnology (M/C 870), University of Illinois, Chicago 60607, USA.) Molecular microbiology, (1999 Mar) 31 (5) 1549-59. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

L59 ANSWER 21 OF 47 MEDLINE on STN DUPLICATE 8
1999148268. PubMed ID: 10025401. Structural basis of multidrug recognition by BmrR, a transcription activator of a multidrug **transporter.**
Zheleznova E E; Markham P N; Neyfakh A A; Brennan R G. (Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland 97201-3098, USA.) Cell, (1999 Feb 5) 96 (3) 353-62. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

L59 ANSWER 22 OF 47 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN
1999:379404 The Genuine Article (R) Number: 195BV. Identification of a new locus in Listeria monocytogenes involved in cellobiose-dependent repression of hly expression. Huillet E; Larpin S; Pardon P (Reprint); Berche P. INRA, PATHOL INFECT & IMMUNOL LAB, F-37380 NOUZILLY, FRANCE (Reprint); INRA, PATHOL INFECT & IMMUNOL LAB, F-37380 NOUZILLY, FRANCE; INSERM, U411, FAC MED NECKER ENFANTS MALAD, F-75015 PARIS, FRANCE. FEMS MICROBIOLOGY LETTERS (15 MAY 1999) Vol. 174, No. 2, pp. 265-272. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS . ISSN: 0378-1097. Pub. country: FRANCE. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L59 ANSWER 23 OF 47 MEDLINE on STN DUPLICATE 9
1999171155. PubMed ID: 10071761. Genomic organization and mutational analysis of the human UCP2 gene, a prime candidate gene for human obesity. Lentes K U; Tu N; Chen H; Winnikes U; Reinert I; Marmann G; Pirke K M.

(Laboratory of Molecular Neurogenetics, University of Trier, Germany.) Journal of receptor and signal transduction research, (1999 Jan-Jul) 19 (1-4) 229-44. Journal code: 9509432. ISSN: 1079-9893. Pub. country: United States. Language: English.

L59 ANSWER 24 OF 47 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN

2000:462187 Document No.: PREV200000462187. Identification of multiple multidrug **transporters** in a novel ciprofloxacin-resistant mutant of Staphylococcus aureus. Simjee, S. [Reprint author]; Shaw, K. J.; Everett, M. J. [Reprint author]; Jin, Y. F. [Reprint author]; Piddock, L. J. V. [Reprint author]. Med. Sch., Div. of Immunity and Infection, Univ. of Birmingham, Birmingham, UK. Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (1999) Vol. 39, pp. 106. cd-rom. Meeting Info.: 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco, California, USA. September 26-29, 1999. American Society for Microbiology.
Language: English.

L59 ANSWER 25 OF 47 CAPLUS COPYRIGHT 2005 ACS on STN

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of biological chemistry, (1998 Oct 9) 273 (41) 26447-54. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

L61 ANSWER 22 OF 34 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN
1998:10568 Document No.: PREV199800010568. Glutamate residues located within putative transmembrane helices are essential for **TetA** (P)-mediated tetracycline efflux. Kennan, Ruth M. [Reprint author]; McMurry, Laura M.; Levy, Stuart B.; Rood, Julian I.. Dep. Microbiol., Monash Univ., Clayton, VIC 3168, Australia. Journal of Bacteriology, (Nov., 1997) Vol. 179, No. 22, pp. 7011-7015. print.
CODEN: JOBAAY. ISSN: 0021-9193. Language: English.

L61 ANSWER 23 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
1996:290658 Document No. 124:315061 Polyspecific immunoconjugates and antibody composites for targeting the multidrug resistant phenotype. Goldenberg, David M. (Immunomedics, Inc., USA). PCT Int. Appl. WO 9604313 A1 19960215, 83 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US9491 19950801. PRIORITY: US 1994-286430 19940805.

L61 ANSWER 24 OF 34 MEDLINE on STN DUPLICATE 11
97121289. PubMed ID: 8961955. Determination of a transmembrane segment using cysteine-scanning mutants of transposon Tn10-encoded metal-tetracycline/H+ antiporter. Kimura T; Suzuki M; Sawai T; Yamaguchi A. (Department of Cell Membrane Biology, Osaka University, Japan.) Biochemistry, (1996 Dec 10) 35 (49) 15896-9. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

L61 ANSWER 25 OF 34 MEDLINE on STN DUPLICATE 12
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L61 ANSWER 26 OF 34 MEDLINE on STN DUPLICATE 13
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L61 ANSWER 27 OF 34 CAPLUS COPYRIGHT 2005 ACS on STN
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L61 ANSWER 28 OF 34 MEDLINE on STN DUPLICATE 14
96064673. PubMed ID: 7592810. The Lactococcal lmrP gene encodes a proton motive force-dependent drug **transporter.** Bolhuis H; Poelarends G; van Veen H W; Poolman B; Driessen A J; Konings W N. (Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute,

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     University of Groningen, Haren, The Netherlands. ) Journal of biological
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     0021-9258. Pub. country: United States. Language: English.

L61  ANSWER 29 OF 34  SCISEARCH  COPYRIGHT (c) 2005 The Thomson Corporation.
     on STN
95:689693   The Genuine Article (R) Number: RY054. THE NH2-TERMINAL HALF OF THE
     TN10-SPECIFIED TETRACYCLINE EFFLUX PROTEIN TETA CONTAINS A
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     MED, CTR ADAPTAT GENET & DRUG RESISTANCE, BOSTON, MA, 02111 (Reprint);
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     JOURNAL OF BIOLOGICAL CHEMISTRY (29 SEP 1995) Vol. 270, No. 39, pp.
     22752-22757. ISSN: 0021-9258. Pub. country: USA. Language: ENGLISH.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

L61  ANSWER 30 OF 34     MEDLINE on STN                    DUPLICATE 15
95362692.     PubMed ID: 7635843.    Tetracycline/H+ antiport and Na+/H+ antiport
     catalyzed by the Bacillus subtilis TetA(L) transporter
     expressed in Escherichia coli. Guffanti A A; Krulwich T A. (Department of
     Biochemistry, Mount Sinai School of Medicine of CUNY, New York 10029, USA.
     ) Journal of bacteriology, (1995 Aug) 177 (15) 4557-61.  Journal code:
     2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

L61  ANSWER 31 OF 34     MEDLINE on STN                    DUPLICATE 16
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     transporter from pACYC184 complements K+ uptake activity in K+
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     Nakamura T; Matsuba Y; Ishihara A; Kitagawa T; Suzuki F; Unemoto T.
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     country: Japan. Language: English.

L61  ANSWER 32 OF 34     MEDLINE on STN                    DUPLICATE 17
96360487.     PubMed ID: 8747276.    Mutational analysis and molecular modelling
     of an amino acid sequence motif conserved in antiporters but not
     symporters in a transporter superfamily. Varela M F; Sansom C E;
     Griffith J K. (Department of Biochemistry, University of New Mexico School
     of Medicine, Albuquerque 87131, USA. ) Molecular membrane biology, (1995
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     country: ENGLAND: United Kingdom. Language: English.

L61  ANSWER 33 OF 34  CAPLUS  COPYRIGHT 2005 ACS on STN
1995:541072   Document No. 122:310317  Gene engineering studies on the
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     Date 1994, 11, 147-53 (Japanese) 1995.  CODEN: YAKSEY.  ISSN: 0914-4544.
     Publisher: Yakugaku Kenkyu Shorei Zaidan.

L61  ANSWER 34 OF 34     MEDLINE on STN                    DUPLICATE 18
93319254.     PubMed ID: 7916584.    Nucleotide and deduced protein sequences of
     the class D tetracycline resistance determinant: relationship to other
     antimicrobial transport proteins. Varela M F; Griffith J K. (Department of
     Cell Biology, School of Medicine, University of New Mexico, Albuquerque
     87131. ) Antimicrobial agents and chemotherapy, (1993 Jun) 37 (6) 1253-8.
     Journal code: 0315061. ISSN: 0066-4804. Pub. country: United States.
     Language: English.


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L63  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2005 ACS on STN
2000:176017  Document No. 132:219218  Diagnosis of multidrug resistance in
     cancer and infectious lesions using immunoconjugates.  Goldenberg, David
     M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 2000014537 A2 20000316,
     40 pp.  DESIGNATED STATES: W:  AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY,
     CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GD, HR, HU, ID,
     IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG,
     MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
     TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ,
     TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1999-US20017 19990901.  PRIORITY: US
     1998-PV99304 19980904.
AB   Immunoconjugates of a diagnostic agent and an antibody component that
     binds an epitope of a multidrug transporter protein are
     disclosed.  Thesse immunoconjugates are used in in vivo diagnostic methods
     to determine whether the failure of traditional chemotherapy is due to the
     presence of multidrug resistant tumor cells, multidrug resistant
     HIV-infected cells or multidrug resistant infectious agents.

L63  ANSWER 2 OF 4  CAPLUS  COPYRIGHT 2005 ACS on STN
1996:290658  Document No. 124:315061  Polyspecific immunoconjugates and
     antibody composites for targeting the multidrug resistant phenotype.
     Goldenberg, David M. (Immunomedics, Inc., USA).  PCT Int. Appl. WO 9604313
     A1 19960215, 83 pp.  DESIGNATED STATES: W:  AM, AT, AU, BB, BG, BR, BY,
     CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR,
     KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, TJ, TM, TT; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE,
     DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD,
     TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1995-US9491 19950801.
     PRIORITY: US 1994-286430 19940805.
AB   Polyspecific immunoconjugates and antibody composites that bind a
     multidrug transporter protein and an antigen associated with a
     tumor or infectious agent are used to overcom the multidrug resistant
     phenotype.  These immunoconjugates and composites also can be used
     diagnostically to determine whether the failure of traditional chemotherapy is
     due to the presence of multidrug resistant tumor cells, multidrug
     resistant HIV-infected cells or multidrug resistant infectious agents.  In
     example, polyspecific immunoconjugates containing G-CSF, 90Yttrium,
     doxorubicin, dextran and anti-P-glycoprotein/anti-carcinoembryonic antigen
     bispecific antibody were prepared for colon cancer treatment.  99mTc- and
     111Indium-labeled polyspecific immunoconjugate targeted to multidrug
     resistant Pseudomonas aeruginosa were also prepared

L63  ANSWER 3 OF 4  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN                                              DUPLICATE 1
96101292  EMBASE  Document No.: 1996101292. Efflux transporters of
     antibiotics in Gram(-) bacteria. A mechanism of multiresistance. Charvalou
     E.. University of Krete, Medical School, Dept. of
     Bacteriol./Parasitol./Zoon.,Rethymnon, Greece. Acta Microbiologica
     Hellenica  40/6 (480-486)  1995.
     ISSN: 0438-9573. CODEN: AMBHAA. Pub. Country: Greece. Language: Greek.
     Summary Language: Greek; English.
AB   Bacterial multidrug resistant strains consists an increasing threat to
     human health. Classical mechanisms of resistance include enzymatic
     degradation and modification of drugs (e.g. hydrolysis of b-lactamases and
     phosphorylation of aminoglycosides), alteration of the antibiotic target
     (e.g. production of modified PBP in MRSA Staph. aureus) and active efflux
     of specific classes of antibiotics (e.g. pumping out of tetracycline by
     Tet proteins). Recently, it has been recognised that active efflux protein
     systems have a special structure spanning the outer and inner membrane in
     Gram(-) bacteria and a surprisingly wide specificity of substrates; such a
     protein-complex identified in Pseudomonas aeruginosa (MexAB-OprK
     ) is contributing in intrinsic resistance of the organism mentioned above.
     In Gram negative bacteria active efflux pumps of broad specificity are
     essential for survival. In Escherichia coli such a system is named Acr and
     it is involved in resistance to dyes, detergents and antibiotics.

L63  ANSWER 4 OF 4     MEDLINE on STN                    DUPLICATE 2
96092505.     PubMed ID: 8549276.   Quinolone mode of action. Hooper D C.
     (Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical
     School, Boston, USA. ) Drugs, (1995) 49 Suppl 2 10-5.  Ref: 32. Journal
     code: 7600076. ISSN: 0012-6667. Pub. country: New Zealand. Language:
     English.
AB   Physical studies have further defined interactions of quinolones with
     their principal target, DNA gyrase.  The binding of quinolones to the DNA
     gyrase-DNA complex suggests 2 possible binding sites of differing
     affinities.  Mutations in either the gyrase A gene (gyrA) or the gyrase B
     gene (gyrB) that affect quinolone susceptibility also affect drug binding,
     with resistance mutations causing decreased binding and
     hypersusceptibility mutations causing increased binding.  Combinations of
     mutations in both GyrA and GyrB have further demonstrated the contribution
     of both subunits to the quinolone sensitivity of intact bacteria and
     purified DNA gyrase.  A working model postulates initial binding of
     quinolones to proximate sites on GyrA and GyrB.  This initial binding then
     produces conformational changes that expose additional binding sites,
     possibly involving DNA.  Quinolones also inhibit the activities of
     Escherichia coli topoisomerase IV (encoded by the parC and parE genes),
     but at concentrations higher than those inhibiting DNA gyrase.  The
     patterns of resistance mutations in gryA and parC suggest that
     topoisomerase IV may be a secondary drug target in E. coli and Neisseria
     gonorrhoeae.  In contrast, in Staphylococcus aureus these patterns suggest
     that topoisomerase IV may be a primary target of quinolone action.
     Regulation of expression of membrane efflux transporters may
     contribute to quinolone susceptibility in both Gram-positive and
     Gram-negative bacteria.  The substrate profile of the NorA efflux
     transporter of S. aureus correlates with the extent to which the
     activity of quinolone substrates is affected by overexpression of NorA.
     In addition, the Emr transporter of E. coli affects
     susceptibility to nalidixic acid, and the MexAB OprK transport
     system of Pseudomonas aeruginosa affects susceptibility to
     ciprofloxacin.(ABSTRACT TRUNCATED AT 250 WORDS)


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